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COLLECTED WORKS ON RADIOBIOLOGY

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Editor

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FOREWORD

The adaptation to practice of the production of atomic energy has opened to mankind the possibility of utilizing for peaceful purposes its boundless resources. The fulfillment of this possibility has been brilliantly effected in our country where there has been established the first electric power station in the world which operates by means of atomic energy.

In addition to this most important performance, nuclear radiations have already found extensive utilization in a number of other domains of human activity. Thus, in medicine there are methods based on the use of penetrating rays for the diagnosis of diseases. In addition, for the purpose of the therapy of a number of disorders, more extensive use is steadily being made of external irradiation with different forms of radiations. Likewise, the introduction into the organism of natural and artificially produced radioactive substances has found application.

Ionizing radiations are extensively utilized in the most diversified fields of scientific research and, in particular, in biology, medicine, agrology, and agronomy. The method of tagged atoms makes it possible to solve rapidly and accurately many scientific problems, and in a number of instances it is only as a result of the use of this new method that their experimental solution has become possible.

Utilization of various kinds of penetrating radiations has found no less application in engineering -- in checking the quality of production (flaw detection) in particular.

From the foregoing it is apparent that at the present time man encounters with steadily increasing frequency various types of radioactive radiations. It is beyond doubt that on further progress in the production and utilization of atomic energy an increase will also be effected in the contingents of persons who, due to the nature of their work, are forced to come in contact with nuclear radiations.

It is well known that one of the characteristic features of ionizing radiations is their high biological activity. In the presence of relatively high degrees of exposure of the animal and human organism, these radiations affect all the organic functions inducing the so-called radiation damage. Therefore, it is entirely natural that a steadily increasing interest is being shown the study of the biological action of ionizing radiations, the evolvement of methods and means for biological protection of the organism, and the therapy of radiation-induced disorders. Moreover, cognizance of the regularities of biological action of radiations is also necessary for the development of the most effective methods of radiation exposure in the treatment of various diseases (neoplasms, et al).

Although the study of the effects of ionizing radiations upon the organism was imitiated a long time ago -- practically speaking, since the discovery of Xrays -- it must be admitted that at the present time science still lacks a satisfactory general theory of the biological action of penetrating radiations. Furthermore, the factual data accumulated are insufficient for the solution of a number of important problems which have arisen in connection with the use of nuclear energy. Hence, the necessity of further studies of the action upon the organism of different types of radioactive radiations is fully evident.

The symposium being hereby submitted to the reader includes a series of experimental papers concerned with two problems: (1) study of the mechanism of the action of certain substances which safeguard the organism from the harmful effects of ionizing radiations; (2) analysis of the action of the penetrating radiations on the fertility of mammals.

Among the substances tested as protective agents, special attention has been given to elucidation of the action of estrogens. The correlation between their protective action and the dose and time of administration has been ascertained, as well as the physiological condition of the thus-protected animal. The conditions whereby the protective action of estrogens is prolonged have been elucidated.

In addition to studies of the protective action of estrogens, a special investigation has been carried out on the effects of implantations of spleen and injection of homologous bone marrow, in the case of irradiated animals. In both cases the beneficial effect of these procedures upon the course of radiation reaction and the survival of the animals has been ascertained.

In three papers the results of studies of the influence of ionizing radiations (single exposure to Xrays and chronic gamma-ray irradiation) on mice fertility are presented. Analysis of fertility was carried out by the method of breeding the irradiated animals to nonirradiated, as well as by means of histological studies of the sex glands of animals exposed to radiations. Embryological studies were also made of the offspring of irradiated parents. Finally, an

analysis of the fertility of irradiated females during their oestrous cycle was studied. In these papers new facts are presented which embody the characteristics of the sterilizing action of ionizing radiations in the exposed animals and their offspring.

The above-enumerated papers are preceded by a contribution in which data are presented on the correlation between the irradiation reaction of mice and the over-all degree of exposure to Xray irradiation. This contribution consiituted the indispensable prerequisite for all the subsequent researches which we conducted on mice.

The papers being published i the present symposium constitute only a portion of the investigation on the effects of ionizing radiations carried out by a team of associates of the cytological laboratory of the Institute of Genetics of the Academy of Sciences USSR (N. I. Nuzhdin, O. N. Petrova, O. N. Kitayeva, M. V. Volkovich, and I. A. Nechayer), and of the radiobiological laboratory of the Institute of Biophysics of the Academy of Sciences USSR (N. I. Shapiro, A. M. Kuzin and Ye. N. Kolodiy).

THE EFFECT OF DIFFERENT DOSAGES OF X-RAY IRRADIATIONS ON THE SURVIVAL OF MICE

N. I. Shapiro and N. I. Nuzhdin

In recent years the attention of biologists has been attracted to a steadily increasing extent by questions concerning the action of ionizing radiations on animal and plant organisms. This interest is due not only to the significance of the radiation method as concerns the study of the structures and properties of living matter, not only to the fact that ionizing radiations are utilized in medicine on a wider scale from one year to the next, but primarily and mostly to the prospects which often open in connection with the possibility of utilizing atomic energy.

Although study of biological action of ionizing radiations was initiated a long time ago and a vast amount of factual data has been accumulated in this field, it must nevertheless be admitted that at the present time we have not only no satisfactory general theory of the biological action of radiation, but a considerable portion of the factual data is deficient and therefore cannot always be utilized.

The unsatisfactory nature of a greater portion of radiobiological researches, especially the early ones, is due primarily to inadequate, or even totally lacking, data as concerns therapeutic exposures. In addition to dosimetric deficiencies in most of the researches, no determinations were made of the indespensable quantitative aspects of the biological action of radiation. Moreover, if we consider that many of the problems which are of immediate concern at the present time were not studied heretofore, it becomes evident why no extensive use can be made of the earlier data and why it becomes necessary to carry out an intensive study of the characteristics of the biological action of ionizing radiations.

In our investigations the task involved was a study of the characteristics of a large number of biological reactions induced as a result of the over-all (total) irradiation of the organism. In the course thereof not only the changes produced in specimens directly exposed to radiations, but also those brought about in their offspring. Special attention has been given to attempts made in regard an active interference with the course of biological reactions induced by the action of ionizing radiation. To find means for controlling these reactions; thus, to learn how the deleterious sequelae of irradiation can be precluded unquestionably constitutes one of the most important

problems in current radiobiology.

The present investigation is concerned with the determinations of quantitative characteristics in the death rate of mice as determined by exposure to Xrays. Such an investigation was a necessary prerequisite for any other, primarily for purely procedural reasons.

Since mice were the principal object of our investigations and the main form of radiation used in the experiments was roentgenological, it was necessary to determine first of all the correlation between the death rate of the experimental animals and the amount of exposure to Xray irradiation. The literature data which are pertinent thereto (Ellinger, 1945; Quastler, 1945) cannot serve in lieu of one's own experimental materials, since the results of investigations of this nature depend upon the strain of mice utilized, their feeding and maintenance conditions, and, finally, on the accuracy of the dosimetric measurements carried out in conjunction with the irradiation experiments.

As was stated before, the present communication is concerned with elucidation of the nature of the relationship which exists between the death rate of mice and the amount of radiation exposure, the determination of mean lethal dose (the dosage which brings about the death of 50% of the experimental animals within 30 days -- LD 50/30), as well as a determination of the life span of the animals and the course of radiation damage in the presence of various degrees of irradiation exposure.

Materials and Procedures

The work was carried out with laboratory white mice of stain A (this stain is characterized by the fact that the females develop cancer

of the mammary glands at the age of 6 months, or later). Males 2 1/2 to 3 months old, weighing from 18 to 23 g, were subjected to irradiation. Prior to irradiation, as well as subsequent thereto, the mice were kept in 10-lit glass jars. Each jar contained 6 mice. The daily ration of each mouse consisted of 2 g of wheat grits, 2 g of white bread, and 5 g of milk. Oats and water were invariably kept before the animals. Once a week the animals were fed a small amount of hard-boiled eggs and carrots.

Irradiation was carried out under the following conditions: voltage: 160 kv; current intensity: 5 ma; filters: cu 0.5 mm, Al 0.75 mm; focal length: 40 cm; dosage 12.9 r/min. The animals were irradiated in groups of 12 in a specially designed box (25 cm x 32 cm x 3.5 cm). Inside the box were 12 compartments in which the animals were placed. Each compartment had the following dimensions: width 3 cm, length 7 cm, height 4 cm. Thus, the mice were distributed not over the entire box, but within a limited area of about 300 cm^2. At the sides of the compartments were placed small bags with mice (20 g). These were provided in order that the mice held in the outer compartments be exposed to about the same amount of diffused radiation as the animals held in the middle compartments.

For 30 days after irradiation the mice were kept under observation and weighed at regular intervals. A portion of those that died were dissected.

Results Obtained and Their Discussion

An investigation was made of the effects of the following doses of Xrays on the vitality of male mice of stain A; 200r, 300r, 350r, 400r, 500r, 600r, 700r,900r, 1100r, 1300r, 1800r, and 2300r, The results

obtained are shown in Table 1.

As is apparent from the data of Table 1, the mortality of mice increases with increasing dosage of Xray irradiation. Beginning with a dose of 700r and greater, death of all the animals results, and a characteristic sharp increase in the mortality rate is observed within the dosage range of 550-600r.

The empirically determined correlations between the percentage of death in mice and the irradiation dosage is in good agreement with an S-shaped curve, the equation of which is

$$y = \frac{100}{1 + e^{5.2597-0.01254x}},$$

wherein y is the percent of animals that died; x - the dosage of Xray irradiation; e the basis of natural logarithms. Figure 1 shows this curve. The lines extending up and down from the empirical points represent the value of a single mean error.

By using the above-stated equation of the curve we have calculated the value of the mean lethal dose LD 50/30. It was found to be 390r.

It is of interest to make a comparison of LD 50/30, which we calculated in the case of mice, with the data on LD 50/30 found in the literature in reference to other mammals. It is true that such a comparison is of somewhat relative nature, since data on injury to animals by different doses of ionizing radiations depend to a considerable extent upon various circumstances (dosimetric accuracy, different irradiation techniques which are of special importance in experiments on large animals, physiological conditions of the experimental animals, and many others).

TABLE 1

CORRELATION BETWEEN DEATH RATE IN MICE AND DOSAGE OF X-RAY IRRADIATION

Dosage (in r)	Total number of animals	Animals which survived		Animals which died	
		number	percent	number	percent
200	31	29	93.1 ± 4.5	2	6.9 ± 4.5
300	24	22	91.8 ± 5.6	2	8.2 ± 5.6
350	22	16	72.7 ± 9.5	6	27.3 ± 9.5
400	64	31	48.4 ± 6.2	33	51.6 ± 6.2
500	47	15	31.9 ± 6.8	32	68.1 ± 6.8
600	43	4	9.4 ± 4.4	39	90.6 ± 4.4
700	12	0	0	12	100
900	12	0	0	12	100
1100	12	0	0	12	100
1300	11	0	0	11	100
1800	12	0	0	12	100
2300	9	0	0	9	100

Notwithstanding the difficulties involved in such a comparison, it still provides certain information concerning the relative radiosensitivity of different species of animals. According to literary data, the mean lethal dose in dogs is within the range of 300-350r (Brecher and Cronkite, 1951); in pigs, 275r (Tullis, Tessmer, Cronkite and Chambers, 1949); in guinea pigs, 230r (Ellinger, 1945); and in rats, 640r (Clark and Uncapher, 1949). Thus, as concerns radiosensitivity, the mice occupy an intermediate position between dogs and rats.

The data which we have obtained not only permit us to determine the quantitative characteristics of the death rate in mice in correlation to the irradiation dosage, but also to ascertain the changes which occur in the course thereof in the life span of the mice. The corresponding data shown in Table 2 reveal that in the presence of increasing dosage of Xray irradiation, the life span of the mice is decreased. This is reflected in the changes of mean life span, as well as in the distribution of daily mortality during the observation period, starting from the time of irradiation.

(See Table 2 on Page 11)

These characteristics are revealed most clearly in a graphic representation of the data derived.

Figure 2 shows the empirical curve which represents changes in the mean life span of the mice, depending on the irradiation dosage.

TABLE 2

LIFE SPAN OF MICE WHICH DIED AFTER SUBJECTION TO VARIOUS DOSES OF ROENTGENOLOGIC IRRADIATION

Dosage (in r)	Total number of animals	Number of animals which died	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean life span (in days)
200	31	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	14.5
300	24	2	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5
350	22	6	-	-	-	-	-	-	1	-	2	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.5
400	64	33	-	-	-	-	-	1	1	6	8	4	6	-	2	1	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.9
500	47	32	-	-	-	-	1	-	2	1	2	8	7	3	3	1	2	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	9.6
600	43	39	-	-	-	1	2	2	4	12	6	5	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.1
700	12	12	-	-	-	-	1	-	4	4	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.3
900	12	12	-	-	-	1	-	6	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.9
1100	12	12	-	-	-	8	-	1	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.7
1300	11	11	-	-	-	10	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.7
1800	12	12	-	-	2	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3
2300	9	9	-	-	2	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3

As the dosage of Xray irradiation increases, the life span decreases up to a certain limit. Within the range of the maximum dosages used by us (1,300 to 2,300 r), the life span of the mice remains practically constant. It would be erroneous, of course, to deduce therefrom that further increase in the dosage will not result in a still greater decrease in life span. There are no reasons for such a conclusion, and, moreover, there are data which support the definite assertion that heavier dosages will reduce the life span of the animal to such an extent that an instance of so-called death due to radiation exposure will result (quastler, 1945). The temporary arrest in the decrease of animal life span in the presence of increase of the dosage of irradiation can, in our opinion, be quite rationally explained. In the presence of radiation injury -- even that due to heavy, lethal dosage -- not all the vitally important systems of the organism are destroyed. Only on exposure to considerably greater doses (possibly of the order of several tens of thousands, and even several hundred thousand r) will the other systems which had previously remained relatively unaffected be destroyed. Thus, the correlation between length of survival of mice and the dosage of Xray irradiation, which we have determined, is determined by radiation damage to sufficiently radiosensitive systems of the organism. Unfortunately at the present time we are not in a position to state specifically which systems are involved in the first and in the second instance.

Figure 3 shows the curves which represent the dynamics of distribution of mortality among the animals during the month following exposure to different doses of Xrays. On examining these curves, one can first of all deduce that the distribution of mortality is sufficiently

specific for each irradiation dosage. Thus, as the dosage increases, the temporal distribution of mortality among the animals, becomes on one hand more compact, i.e., less spread out, and on the other, shifted to points of time steadily more proximate to the time of irradiation.

In connection with the discussion of the time of death of the animals following irradiation, let us consider the question as to whether there exists a correlation between the initial weight of the mice and their fate after irradiation. It has been reported in the literature that there exists a negative correlation between the weight of the animal and its radiosensitivity, i.e., statements to the effect that upon a given dosage of irradiation there is a greater probability of the death of mice of lesser weight (Quastler, 1945). The materials which we had at our disposal could be utilized for an experimental verification of this contention.

One hundred and nine male mice of strain A, aged 2 to 3 months, were irradiated with a dosage of 500r (the conditions of exposure have been stated above). The weight of the experimental animals varied from 19 to 28 g. If some correlation actually exists between the initial weight of the irradiated animal and its subsequent fate, the mean initial weight of the mice that died as a result of exposure to radiation must differ from the mean initial weight of the mice that survived. After the necessary calculations, the following results were obtained.

M_I (mean initial weight, computed on the basis of the weight of 36 mice which survived after irradiation) = 24.30 ± 0.28 g.

M_{II} (mean initial weight, computed on the basis of the weight of 73 mice which died after irradiation) = 23.73 ± 0.22 g.

Hence,

$$M_{dif} = 0.57 \pm 0.36 \text{ g}.$$

In other words, the difference between M_I and M_{II} was found to be statistically unreliable. Thus, the data obtained support the assertion that the initial weight of the animal does not determine its fate following irradiation, and that consequently the probability of death due to irradiation of leaner mice is not greater than that of fatter mice.

However, the lack of a correlation between the initial weight of the animals and their death or survival after irradiation does not by any means indicate the absence of a correlation between the initial weight and the course of radiation injury. In particular, it was not known whether there exists a correlation between the initial weight of mice and the duration of their life after irradiation. To resolve this question we have made an analysis of the material relating to the same 73 mice which died as a result of radiation injury. Their time of death varied from 3 to 29 days after irradiation. A correlation was calculated between the initial weight and the life duration of these mice. The coefficient of correlation was found to be so small ($r = -0.05 \pm 0.05$) that there are no reasons whatever for assuming the existence of any relationship between the initial weight of the experimental animals and the duration of their life after irradiation.

As was stated herein before, in addition to quantitative determinations of the death rate of animals at different dosages of irradiation, we have carried out systematic observations on the course of radiation injury in these animals. In these observations changes were recorded in the outward appearance of the animals, the behavior of the animals, and other clinical symptoms as well.

Essentially, the symptoms of radiation injury in mice are the same in the presence of all the irradiation dosages which we have studied. In these instances, as in any other biological reaction induced by ionizing radiation, the extent of manifestation of the changes increases with increasing irradiation dosage. We will present as an example the description of the disorders in animals subjected to an irradiation dosage of 500r.

During the first 2 days after irradiation no deviations from the normal are observed. On the fifth to sixth day the animals become sluggish, appreciably thinner and weaker, and some develop diarrhea. The appetite decreases sharply, and at the same time thirst increases drastically. The appearance and behavior of some of the mice is very characteristic during the critical stage of radiation injury. The back of the animal is strongly arched; it remains motionless with ruffled fur which is unkept and soiled. Reactions to external stimuli are weak. The gait is slow and waddling. The first instances of death following exposure to this dose occur as a rule on the sixth to seventh day and reach their maximum on the ninth to eleventh. After the 15th to 17th days, instances of death as a result of radiation injury practically cease to occur. Isolated cases of mice which die later obviously succumb to secondary infection (Bacteriemia). Changes in weight of the experimental animals provide a good indication of the course of radiation injury (Figure 4). The numerical data on the basis of which the weight-variations curves are plotted are shown in Tables 3 and 4 (appearing at the end of the present paper.)

For a ready comparison use was made not of the absolute weight values of the animals, but of indices (ratio of mean weight of the animals on a

given day to the mean weight on the day of irradiation).

On comparing the curves of the weight-index variation of mice which survived after irradiation, it is apparent that with increasing dose of radiation exposure the difference between the experimental group of animals (which survived after irradiation) and the controls increases. This difference manifests itself essentially along two lines. First, over the entire period of observations the weight of irradiated mice is found to be below that of the controls. The lag in weight becomes clearly manifest on irradiation with a dose as low as 200r and reaches its maximum with a dose of 600r. Secondly, the difference resides in the very shape of the curves. The controls show a steady increase in weight, whereas the experimental group, beginning with a dosage of 300r, reveals first a decrease and only thereafter a decrease in weight. The extent of decrease in weight is proportional to the exposure dosage: thus, decrease in weight is least in the case of an exposure of 300r and greatest in the case of 600r. In the case of a dosage of 200r, no decrease in weight is observed. Over a fairly long period the weight remains at about the same level. Furthermore, the weight variation curves of irradiated animals differ from one another in the time where the minimum weight is reached. Thus, in the case of a dose of 300r, the minimum in weight is observed on the sixth day, in the case of 400 to 500r on the eleventh to twelfth day, and in the case of a dose of 600r, on the thirteenth to fifteenth day following irradiation. These shifts are evidently associated with different time intervals which precede the onset of recuperative processes in the irradiated organism. The recuperative processes begin sooner and overcome more rapidly the disintegration processes in mice which have been exposed to lesser doses of Xrays.

Weight variation curves of the animals which died after irradiation show a very sharp decrease in weight occurring in the presence of all dosages of exposure.

A more detailed study of changes in the weight of animals which died and those which survived, taking place during the first few days following irradiation, may possibly permit the use of weight indices for prognosticative purposes.

Conclusions

1. The quantitative characteristics of death in mice following total irradiation with different dosages of Xrays are represented by an S-shaped curve, corresponding to the equation

$$y = \frac{100}{1 + e^{5.2597-0.01254x}},$$

wherein y is the percent of mortality among the irradiated animals, x the dose of exposure, and e the basis of natural logarithms.

2. Life duration of mice following irradiation decreases from 14.5 days to 3.3 days with increasing dose of exposure (from 200 to 2300r).

3. It has been shown that there is no correlation between the initial weight of sexually mature animals and their life duration or death following irradiation.

4. Analysis of the course of radiation injury in mice shows that the symptoms of this disorder are qualitatively indentical at all the tested doses of exposure and differ only in their quantitative manifestation.

5. An objective index of the extent of damage inflicted to animals by ionizing radiation and of the course of the radiation damage is provided by the dynamics of the weight changes of the experimental animals.

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TABLE 3

CHANGES IN WEIGHT OF MICE WHICH SURVIVED FOLLOWING EXPOSURE TO DIFFERENT DOSAGES OF XRAYS. (THE ZERO-DAY IS THAT OF IRRADIATION)

Dosage (in r)	Number of animals	Weight of animals	Day of weighing														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
200	29	Mean weight (in g)	22.1	-	-	22.3	-	-	-	-	22.4	-	-	-	22.6	-	-
		Index	100.0	-	-	100.9	-	-	-	-	101.3	-	-	-	102.3	-	-
300	22	Mean weight (in g)	22.0	22.2	-	21.9	-	-	21.6	-	21.7	-	21.9	-	-	22.7	-
		Index	100.0	100.8	-	99.5	-	-	98.2	-	98.7	-	99.5	-	-	103.3	-
400	31	Mean weight (in g)	22.5	-	-	-	22.3	-	-	-	22.0	-	-	-	21.3	-	-
		Index	100.0	-	-	-	99.0	-	-	-	97.7	-	-	-	94.6	-	-
500	18	Mean weight (in g)	22.2	-	-	20.7	-	-	-	21.5	-	-	-	20.9	-	-	-
		Index	100.0	-	-	93.4	-	-	-	96.8	-	-	-	94.2	-	-	-
600	4	Mean weight (in g)	21.5	-	21.2	-	20.1	-	19.3	-	20.0	-	-	19.2	-	17.7	-
		Index	100.0	-	98.5	-	93.5	-	89.7	-	93.0	-	-	89.2	-	82.3	-
Not irradiated animals	43	Mean weight (in g)	21.9	-	-	-	23.0	-	-	-	23.2	-	-	-	23.5	-	-
		Index	100.0	-	-	-	105.0	-	-	-	106.0	-	-	-	107.4	-	-

Dosage (in r)	Number of animals	Weight of animals	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
200	29	Mean weight (in g)	-	23.7	-	-	-	-	24.3	-	-	-	24.8	-	-	-	25.3
		Index	-	107.3	-	-	-	-	109.9	-	-	-	112.2	-	-	-	114.4
300	22	Mean weight (in g)	23.4	-	23.0	-	-	22.9	-	23.7	-	24.0	-	-	24.5	-	24.9
		Index	106.4	-	104.5	-	-	104.1	-	107.8	-	109.1	-	-	111.4	-	113.2
400	31	Mean weight (in g)	-	22.3	-	-	-	-	23.4	-	-	-	23.5	-	-	-	24.4
		Index	-	99.0	-	-	-	-	102.7	-	-	-	104.3	-	-	-	108.4
500	18	Mean weight (in g)	21.6	-	-	-	-	22.4	-	-	-	-	23.3	-	-	-	24.0
		Index	97.3	-	-	-	-	100.8	-	-	-	-	105.0	-	-	-	108.1
600	4	Mean weight (in g)	17.7	-	17.9	-	-	18.3	-	20.2	-	-	-	-	22.0	-	22.8
		Index	82.3	-	83.3	-	-	85.1	-	93.3	-	-	-	-	102.2	-	106.0
Not irradiated animals	43	Mean weight (in g)	-	24.4	-	-	-	-	25.1	-	-	-	25.7	-	-	-	26.1
		Index	-	111.5	-	-	-	-	114.8	-	-	-	117.3	-	-	-	119.2

TABLE 4

CHANGES IN WEIGHT OF MICE WHICH DIED FOLLOWING EXPOSURE TO DIFFERENT DOSAGES OF XRAYS. (THE ZERO-DAY IS THAT OF IRRADIATION)

Dosage (in r)	Number of animals and their weight	Days of weighing																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
200	Number of animals	2	-	-	-	2	-	-	-	2	-	-	-	-	1	-	-	-	1
	Mean weight (in g)	19.4	-	-	-	18.9	-	-	-	17.8	-	-	-	-	15.1	-	-	-	15.0
	Index	100.0	-	-	-	97.4	-	-	-	91.7	-	-	-	-	77.8	-	-	-	77.3
300	Number of animals	2	2	-	2	-	-	2	-	2	-	2	-	-	-	-	-	-	-
	Mean weight (in g)	20.3	20.6	-	20.5	-	-	19.9	-	19.9	-	19.0	-	-	-	-	-	-	-
	Index	100.0	101.4	-	101.0	-	-	98.0	-	98.0	-	93.5	-	-	-	-	-	-	-
400	Number of animals	21	-	-	-	21	-	-	-	17	-	-	-	3	-	-	-	-	-
	Mean weight (in g)	22.4	-	-	-	21.9	-	-	-	20.8	-	-	-	18.7	-	-	-	-	-
	Index	100.0	-	-	-	97.8	-	-	-	92.8	-	-	-	83.5	-	-	-	-	-
500	Number of animals	33	-	-	33	-	-	-	28	-	-	-	9	-	-	-	-	-	-
	Mean weight (in g)	22.4	-	-	20.3	-	-	-	20.4	-	-	-	17.9	-	-	-	-	-	-
	Index	100.0	-	-	90.7	-	-	-	91.0	-	-	-	79.9	-	-	-	-	-	-
600	Number of animals	39	-	-	-	39	-	29	-	18	-	-	4	-	-	-	-	-	-
	Mean weight (in g)	21.3	-	-	-	19.2	-	18.8	-	17.7	-	-	15.5	-	-	-	-	-	-
	Index	100.0	-	-	-	90.0	-	88.2	-	83.0	-	-	72.6	-	-	-	-	-	-
700	Number of animals	12	12	-	12	-	-	11	-	5	-	-	-	-	-	-	-	-	-
	Mean weight (in g)	22.2	21.8	-	20.4	-	-	17.7	-	17.3	-	-	-	-	-	-	-	-	-
	Index	100.0	98.3	-	92.0	-	-	79.7	-	78.0	-	-	-	-	-	-	-	-	-
900	Number of animals	12	12	-	12	-	-	5	-	-	-	-	-	-	-	-	-	-	-
	Mean weight (in g)	21.9	20.9	-	18.9	-	-	16.0	-	-	-	-	-	-	-	-	-	-	-
	Index	100.0	95.6	-	86.4	-	-	73.1	-	-	-	-	-	-	-	-	-	-	-
1100	Number of animals	12	12	-	-	4	-	3	-	1	-	-	-	-	-	-	-	-	-
	Mean weight (in g)	22.5	21.0	-	-	17.7	-	15.3	-	13.0	-	-	-	-	-	-	-	-	-
	Index	100.0	93.3	-	-	78.6	-	68.0	-	57.8	-	-	-	-	-	-	-	-	-
1300	Number of animals	11	11	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean weight (in g)	21.2	20.4	-	-	16.5	-	-	-	-	-	-	-	-	-	-	-	-	-
	Index	100.0	96.2	-	-	77.8	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 4 (continued)

		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Number of animals	12	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1800	Mean weight (in g)	20.1	-	17.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Index	100.0	-	85.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Number of animals	9	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2300	Mean weight (g)	22.0	-	18.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Index	100.0	-	85.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

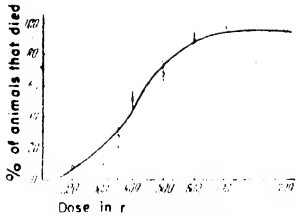

Figure 1. Relationship between death rate in mice and irradiation dosage.

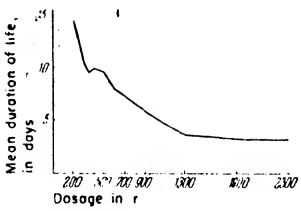

Figure 2. Changes in mean life duration of mice, depending on irradiation dosage.

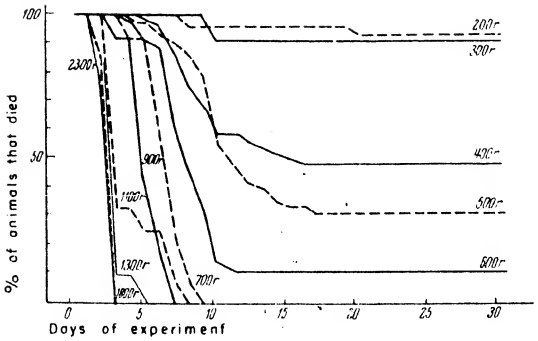

Figure 3. Dynamics of death occurrence in mice following irradiation with various dosages.

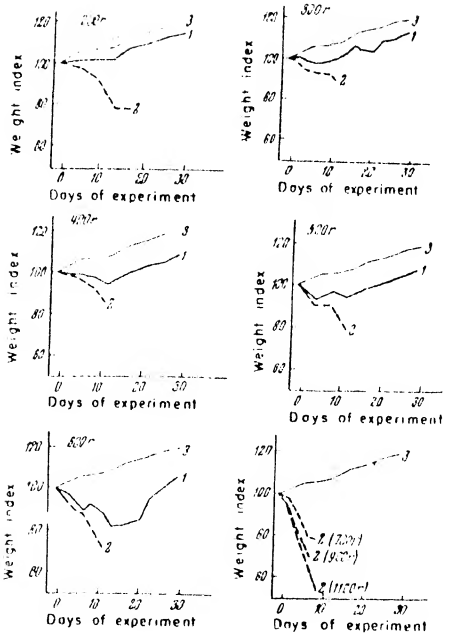

Fig. Change in weight of mice following X-ray irradiation in various doses.

1, control A (surviving); 2, experiment (the mice which died); 3, control.

THE ACTION OF ESTROGENS ON THE RADIATION REACTION IN MICE

N. I. Shapiro, N. I. Nuzhdin, A. M. Kuzin

INTRODUCTION

Researches intended for the elucidation and study of the substances affording protective action against the deleterious effects of ionizing radiations on biological objects are of great theoretical and practical value. It is unquestionable that one of the most promising ways toward the understanding of the mechanism of the action of penetrating radiations upon the organism is the study of conditions which alter the biological effects of these radiations. The capability of altering the biological reactions induced by radiation, and hence also of controlling these reactions, is most important in medical practice in which one is confronted on the one hand with the task of enhancing the efficacy of radiation therapy and on the other of developing a number of health-safeguarding means for the benefit of persons employed at enterprises where use is made of ionizing radiations. The foregoing provides adequate explanation of the vast increase in research concerned with the discovery and investigation of substances which protect the organism from the damaging action of radiation.

It is well known that radiation injury which is induced as a result of a total exposure of the animal to penetrating radiation is actually due to damage inflicted to most, if not all, of its organs. Although we know that the radiosensitivity of the organs differs, there are reasons for believing that with the use of sufficiently massive dosages of irradiation almost all of the vitally important systems are damaged and it is then merely a question of the extent of such damage.

The natural conclusion to be drawn therefrom is that the means for controlling radiation damage must be of a composite nature and consist of a concurrent application of many substances which affect different organs and tissues. The necessity of such a synthetic approach to the problem of finding remedies which afford protection against the damaging effects of radiation does not exclude -- but on the contrary, presupposes -- a search for and a study of the protective properties of individual substances. At the present time the appropriateness of searching for individual protective substances is also being demonstrated by practical attainments. It is sufficient to mention the use for this purpose of antibiotics and blood transfusion. Thus, it is unquestionable that only a combination of an analytic and synthetic approach will ensure a complete resolution of the given problem.

Described in the present communication are the results of experiments on determination of the protective action of estrogens. We have devoted our attention to the estrogens because of their strong effects on the most diversified systems of the animal organism which lead to alterations of the organism's reactivity in the presence of diverse external influences. In particular, the literature contains conflicting reports concerning the role of estrogens in the changes of radiosensitivity of animals. Thus, in 1943 a communication was published in which it was reported that administration to mice of estradiol benzoate prior to irradiation with Xrays increases the resistance of the mice to radiation exposure (Treadwell, Gardner, and Laurence, 1943).

In 1949 two other contributions on this subject were published (Patt, Strande, Tyree, Swift, and Smith, 1949; Graham and Graham, 1949).

In addition to the confirmation of the results of the first communication, in one of these contributions (Graham and Graham, 1949) factual data are presented, and the conclusion is derived to the effect that another estrogen, viz., diethylstilbestrol, is completely devoid of protective properties. As a result, the impression is being conveyed that the protective action constitutes a specific property of estradiol benzoate and not of estrogens in general.

Such a conclusion appeared entirely unsubstantiated in view of general biological concepts. Therefore, it was decided to carry out a special investigation of estrogens as concerns their protective properties. For this purpose, selection was made of diethylstilbestrol and synestrol, which are synthetic estrogens widely utilized in medicine.

Determination of the Existence of the Protective Action of Synestrol and Diethylstilbestrol

In testing the protective action of Synestrol and diethylstilbestrol, the objects of the investigation were male white mice of strain A.

Ten days prior to the Xray irradiation, the animals of the experimental series were subcutaneously administered 0.2 mg of the estrogenic substance dissolved in 0.2 ml of olive oil. The control animals were given only olive oil. The single, general Xray irradiation was carried out under the following conditions: voltage: 160 kv; current intensity 5 ma; filters: 0.75 mm Al + 0.5 mm Cu; focal distance 40 cm; dosage rate 12.9 r/min. Total dosage of exposure was 500r. Irradiation was carried out in wood containers which held 12 mice of which six were of the experimental series (having received the estrogenic substance) and six were of the control series. Since our task was a study of the effects of estrogenic

substances upon the vital stability of the organism in the presence of damage inflicted thereto by large lethal dosages of Xrays, we selected as the basic index the one that was of greatest interest to us, i.e., the survival rate. Observations of the experimental mice were continued for one month after irradiation. Once every 4 days the animals were weighed. Data on the effects of estrogenic substances on the survival rate of the irradiated mice are shown in Table 1.

As is apparent from the tabulated data, both the preparations investigated sharply increase the resistance of mice to the damaging effects of radiation. Synestrol increases the survival rate of the animals about one and a half times. Diethylstilbestrol exhibits a considerably greater protective action, and yielded in all four series of experiments good, well-defined results.

In Table 1 of the addendum (see addendum at the end of the present paper) are shown the summative data on the time of death of the animals following irradiation, for the experimental as well as for the control series. These data reveal that the mean duration of life of the mice which were given estrogen is somewhat less than that of the control animals. Figure 1 shows the curves which represent the time of death of the animals following irradiation.

TABLE 1

THE NUMBER OF MICE THAT SURVIVED AND THOSE THAT DIED IN THE EXPERIMENTAL (ESTROGEN-TREATED) AND THE CONTROL GROUPS

Nature of treatment	No of series and designation		Total number of animals	Animals that survived		Animals that died	
				Number	Percent	Number	Percent
Administration of synestrol	1	Experiment	18	9	50.0	9	50.0
		Control	18	3	16.3	15	83.7
	2	Experiment	30	21	67.9	9	32.1
		Control	36	11	30.6	25	69.4
	3	Experiment	29	14	48.4	15	51.6
		Control	30	15	50.0	15	50.0
	For all series	Experiment	77	44	57.1±5.64	33	42.9±5.64
		Control	84	29	34.5±5.18	55	65.5±5.18
Administration of diethyl-stilbestrol	1	Experiment	24	29	79.2	5	20.8
		Control	20	7	35.0	13	65.0
	2	Experiment	24	23	95.8	1	4.2
		Control	24	9	32.6	15	67.4
	3	Experiment	24	17	70.8	7	29.2
		Control	30	15	50.0	15	50.0
	4	Experiment	17	10	58.9	7	41.1
		Control	18	5	29.8	13	70.2
	for all series	Experiment	89	69	77.5±4.4	20	22.5±4.4
		Control	92	36	39.1±5.1	56	60.9±5.1

The results of observations on the course of radiation injury in mice treated with estrogenic substances and in those not treated are indicative. The radiation injury had a considerably less acute course in the experimental animals than in those of the control group. This was especially apparent in the series wherein diethylstilbestrol was administered. The animals which had been given the estrogen had a better appearance following irradiation than the controls; the behavior of many of them differed but little from that of animals which had not been irradiated. A milder course of the radiation injury in animals which had been given the estrogen is also confirmed by the data relating to their weight, Table 11 of the addendum shows numerical data, and Figure 2 shows the curves which characterize changes in the weight of the animals during 30 days following the administration of estrogen. To characterize the changes in weight of the irradiated animals we used not the absolute values but an index (ratio of mean weight of mice on a given day of observation to the mean weight on the day of irradiation, which is taken as being equal to 100). A comparison of the curves shows that decrease in weight of the animals of the experimental group is somewhat less than that of the animals of the control group.

Still more important is the fact that the animals which had been given the estrogenic substances begin to recover appreciably sooner than the controls. When the mice of the experimental groups begin to gain in weight, those of the control groups still show a decrease in weight. Thus, animals which had been given diethylstilbestrol reach a minimum in weight on the second to seventh day (on the sixth day in the case of Synestrol) after which recovery sets in; among the controls

loss in weight continues to the twelfth to thirteenth day after which a gain in weight begins. The dynamics of changes in weight of the mice following irradiation indicates conclusively that the recuperative processes take place more intensively in animals which had received the estrogen.

For a more complete characteristic of the protective action of diethylstilbestrol, analyses of the peripheral blood of the irradiated animals were carried out. Four groups of animals were used for the blood studies. Ten days prior to irradiation, the mice of the first group (36 animals) were given a subcutaneous injection of 0.2 mg diethylstilbestrol in 0.2 ml of olive oil. The second group (36 animals) were the controls of the first group; these mice received an injection of 0.2 ml sunflower-seed oil 10 days prior to irradiation. Animals of the first and second group were kept under observation for 10 days prior to irradiation and for 47 days thereafter. The third group (16 animals) was used to study the effects of diethylstilbestrol on the blood in the absence of a subsequent irradiation. The fourth group (16 animals) were the controls of the third group, and these animals were given an injection of 0.2 ml olive oil without subsequent irradiation. The animals of the third and fourth groups were kept under observation for 28 days after the injection.

During the course of the experiment, records were kept of the total amount of lenkocytes and erythrocytes, and the percentage of hemoglobin content was determined. Blood for analysis was withdrawn from the caudal vein, and each time in six animals of the first and second groups, and in four animals of the third and fourth groups

(an exception were the thirteenth and twenty-first days following irradiation when three animals were used in each of the experimental and the control group). The hematological changes in animals of all four groups are represented by curves (Figures 3, 4, 5); numerical data are shown in the addendum (Tables 3 and 4).

Because of the fluctuations of the blood-index values in the nonirradiated control animals, it is difficult to make a definite statement concerning the effects of diethylstilbestrol on the blood of nonirradiated animals, but the fact is apparent that the blood indices of animals which had been given the estrogen are in all cases lower than those of the control animals. Maximum decrease of the total number of leukocytes in the irradiated animals was observed on the fifth day following the exposure, and this decrease was less in mice which had been given diethylstilbestrol) than in those of the control group. Maximum decrease in the number of erythrocytes was observed on the ninth day following irradiation, and was also somewhat greater in the control group than in the experimental group. A somewhat-increased number of erythrocytes on the thirteenth day following irradiation in the control group, as compared with the experimental, may be of a fortuitous nature, since at this time only a few animals were investigated (three mice in each control and experimental group, as was stated above).

Restoration of the number of erythrocytes occurred more rapidly in the experimental group than in the control group, attaining its normal level on the twenty-first day following irradiation. At that time the controls still revealed a lagging-behind in relation to the normal values.

Lowering of the hemoglobin content was observed on the ninth to thirteenth day following irradiation, and it was of a lesser extent in the experimental group, wherein its restoration also occurred more rapidly than among the control animals.

In summing up the results of the effects of diethylstilbestrol on the total amount of leukocytes, erythrocytes, and the hemoglobin percentage in the irradiated mice, it can be stated that the lowering of the enumerated indices during the course of radiation injury was less in animals protected by the estrogen than in the control animals. Restoration occurred more rapidly in the experimental group than in the controls (especially as concerned the number of erythrocytes and hemoglobin percentage). Thus the data obtained leave no doubt concerning the positive effects of estrogenic substances, and especially of diethylstilbestrol, on the course of radiation injury.

Correlation Between the Protective Effect and the Dosage of Diethylstilbestrol

To determine the correlation between the survival rate of the animal subjected to Xray irradiation and the amount of estrogenic substance administered to them, we have tested the action of the following dosages of diethylstilbestrol: 0.025, 0.05, 0.1, 0.2, 0.8 mg. The diethylstilbestrol was dissolved in olive oil and administered to the mice subcutaneously 10 days prior to the irradiation (each mouse was given 0.2 ml of the oil solution of the estrogen). The control animals were injected with a corresponding amount of olive oil. The dosage of Xray irradiation was 500 r. The conditions of irradiation, the keeping of the experimental animals, the nature and time intervals of observations were similar to those described above. The results of the experiments are shown in Table 2.

TABLE 2

THE EFFECT OF VARIOUS DOSAGES OD DIETHYLSTILBESTROL FOLLOWING IRRADIATION ON THE SURVIVAL OF MICE

Diethylstilbestrol dosage (in mg)	Experiment					Control				Survival Index (Ratio of % of animals that survived in the experiments to the % of those that survived in the controls
	Animals that survived		Animals that died			Animals that survived		Animals that died		
	Total number of animals	Number	Percent	Number	Percent	Total number of animals	Number	Percent	Number	Percent	
[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
0.025	63	45	71.4±5.6	18	28.6±5.6	54	19	35.2±6.5	35	64.8±6.5	2.03
0.050	89	56	62.9±5.0	33	37.1±5.0	102	29	28.5±4.4	73	71.5±4.4	2.20
0.100	40	31	77.5±6.6	9	22.5±6.6	40	14	35.0±7.4	26	65.0±7.4	2.22
0.200	89	69	77.5±4.4	20	22.5±4.4	92	36	39.1±5.1	56	60.9±5.1	1.98
0.800	33	24	72.7±7.7	9	27.3±7.7	34	9	26.5 7.6	25	73.5±7.6	2.74

Irradiation of experimental and control animals was carried out jointly. Comparison of the protective action of different doses of the estrogen was carried out separately for each of the series. To evaluate the protective action of diethylstilbestrol we used the ratio of the percent of animals that survived in the experiments to the percent of those which survived in the controls. We have designated this ratio the survival index. As is apparent from the data shown in Table 2, all the tested dosages of the preparation produced a considerable protective action, and no correlation is observed between the effectiveness of the action and the amount of diethylstilbestrol that had been administered. In practice, any of the utilized dosages increases about 2 times the survival rate of the irradiated animals. An exception is the dosage of 0.8 mg, which produced a somewhat greater protective action. The fact that the smallest of the tested dosages of the estrogen practically does not differ in its protective action from considerably larger dosages indicates the possibility of a further reduction in the amount of administered diethylstilbestrol without decrease of its efficacy.

The data relating to the life duration of the experimental and control animals which died following irradiation are shown in Table 5 of the addendum, and the corresponding curves in Figure 6.

Observations of the irradiated mice reveal that mice which had been given the estrogen withstand the radiation injury much better.

Figure 7 shows the curves of changes in weight of the mice which survived the radiation injury (numerical data on weight determinations on the basis of which these curves were plotted are shown in Table 6 of the addendum). In this instance, the same as before, not the absolute values

of mean weight are given, but rather the indices (ratio of mean weight of mice on a given day of observation to the mean weight on the day of irradiation, which is taken as being equal to 100).

The curves show that in some instances the weight indices of the experimental mice are better than those of the controls. This is manifested either by an earlier cessation of loss in weight or by a sharper increase of the weight after reaching the minimum value. This is most apparent on comparison of the experimental and control animals in the case of a dosage of 0.2 mg of diethylstilbestrol.

Thus, on summing up, the conclusion can be reached that different doses of diethylstilbestrol protect to a considerable extent the organism of the animal from the lethal effects of ionizing radiation.

Duration of the Protective Action of Diethylstilbestrol

In all of the foregoing experiments the estrogen was administered to the animals 10 days prior to a total Xray irradiation. At the same time it was important to ascertain how soon after the administration of diethylstilbestrol the organism would become more resistant and over what period this resistance would be retained. It was also of interest to determine whether the duration of the action of diethylstilbestrol depends upon the procedure of its administration, and, finally, whether the organism becomes inured to the preparation or whether it is possible to prolong the duration of its action by repeated administration.

The answers to the above-stated questions are of undeniable interest, since they contribute to the elucidation of the physiological pathways upon which this substance affects the organism. The latter is of importance to the resolution of problems concerning the nature of the radiosensitivity

of such highly-developed animals as the mammals.

To determine these questions special experiments were carried out. The objects of the study, conditions of irradiation, and the keeping of the animals were the same as before.

One 3, 5, 10, 12, and 15 days prior to irradiation, the mice of the experimental group were subcutaneously injected with 0.05 mg of diethylstilbestrol each, dissolved in 0.2 ml of refined vegetable oil. The controls were injected with only the oil itself. The experimental results are shown in Table 3.

(See Table 3 on Page 37)

To facilitate a comparison of the efficacy of the action of diethylstilbestrol upon administration at different intervals of time, the survival index, representing the ratio of the percent of animals that survived in the experiments to the percent of animals that survived in the controls, was also calculated.

Analysis of the date of Table 3 shows that the protective action of diethylstilbestrol begins to take effect one to 3 days after administration and terminates between the twelfth and fifteenth day. It is of interest to note that increase, as well as to some extent, decrease, of the protective action of diethylstilbestrol occurs quite rapidly. However, within those intervals of time which were used in the studies, it was not possible to reveal fully the dynamics of this process.

While an administration of the preparation one day prior to irradiation had no effect on the survival rate, administration 3 days prior to irradiation resulted in a full manifestation of the protective action.

TABLE 3

PROTECTIVE ACTION OF DIETHYLSTILBESTROL (0.05 mg) ADMINISTERED TO MICE AT VARIOUS TIMES PRIOR TO IRRADIATION (DOSAGE OF 500r)

Time of administration	Treatment	Total number of animals	Survived		Died		Survival index	Mean duration of life (days)
			Number	Percent	Number	Percent		
1 day prior	Diethylstilbestrol	23	18	78.3	5	21.7	1.2	11.3
	Control	24	16	66.7	8	33.3		14.1
3 days prior	Diethylstilbestrol	24	18	75.0	6	25.0	2.5	15.7
	Control	23	7	30.4	16	69.6		8.7
5 days prior	Diethylstilbestrol	22	16	72.7	6	27.3	2.5	14.3
	Control	24	7	29.2	17	70.8		11.9
10 days prior	Diethylstilbestrol	89	56	62.9	33	37.1	2.2	10.3
	Control	102	29	28.5	73	71.5		9.6
12 days prior	Diethylstilbestrol	48	13	27.1	35	72.9	2.2	11.5
	Control	48	6	12.5	42	87.5		8.8
15 days prior	Diethylstilbestrol	22	11	50.0	11	50.0	1.2	11.0
	Control	22	9	40.9	13	59.1		11.4

Approximately the same picture was also observed as concerns the termination time of the action of the preparation. Effectiveness of the estrogen upon administration 10 to 12 days prior to irradiation was very high, while upon administration 15 days prior to irradiation practically no protective action could be detected.

During those periods when administration of diethylstilbestrol increases the survival rate of the experimental animals, it also increases the mean duration of life of the mice (all things considered, the mice still die as a result of exposure to radiation). Consequently, these data also indicate the favorable action of the estrogen.

It is of interest to consider the dynamics of death occurrence among both experimental and control animals during one month following irradiation. The corresponding data are shown in Table VII of the addendum and by the graphs of Figure 8. The graphs reveal that in those instances when the estrogen is administered 3,5, and 10 days before irradiation there is a sharp difference in the life duration of experimental and control mice. While in the case of the controls most of the deaths occur on the fifth to tenth day following irradiation, in the case of the experimental mice the deaths occur at a later time.

Observations of the course of radiation injury in experimental and control mice indicate a relatively milder course of the disease in animals which had received diethylstilbestrol during the period preceding by 3 to 10 days the irradiation. This can be illustrated by means of the curves which represent changes in the weight of the animals following exposure to Xrays (Figure 9).

The factual data on the basis of which these curves are plotted are shown in Table VIII of the addendum. Characteristic changes in the weight dynamics of the experimental animals are observed on administration of the estrogen 3, 5, and 10 days prior to irradiation. As compared with the controls, the mice of the experimental groups show a gain in weight starting at an earlier date, and, what is of further importance, this increase in weight proceeds at a somewhat higher rate.

On summing up the above-presented data, it can be stated that the protective action of a single subcutaneous administration of an oil solution of diethylstilbestrol manifests itself for about 10 days. It is important to note that within this interval of time the effectiveness of the preparation was found to undergo relatively little change and remained at a constant high level.

As was stated in the introduction, one of the tasks of the present work was a determination of the possibility of increasing the duration of the protective action of the preparation. It is known that the action of estrogens is prolonged upon their subcutaneous introduction into the organism in the form of pellets. In such a case the animal appears to be provided with a supply, so to speak, of diethylstilbestrol, which on gradual resorption brings about an enrichment of the organism with the estrogen. However, heretofore it was not known whether or not it is possible to likewise prolong by this procedure the protective action of the estrogen. To determine this question a special series of experiments was carried out. Pellets containing 0.4 mg diethylstilbestrol in admixture with talc were inserted under the skin of male mice of strain A. The controls received pellets containing no estrogen. Total Xray irradiation of the animals with a dose of 500r was carried out on the

tenth, twentieth, and thirtieth day following the insertion of the pellets. The conditions of irradiation were the same as in the preceding experiments. The results of these experiments are shown in Table 4.

TABLE 4

EFFECTS OF SUBCUTANEOUS INTRODUCTION OF PELLETS CONTAINING DIETHYLSTILBESTROL ON THE SURVIVAL OF MICE FOLLOWING A TOTAL XRAY IRRADIATION (DOSAGE 500r)

Time of introduction of the pellets	Total number of animals	Survived		Died		Mean duration of life (days)
		Number	Percent	Number	Percent	
[1]	[2]	[3]	[4]	[5]	[6]	[7]
10 days prior to irradiation	48	33	68.7	15	31.3	11.0
Control	48	16	33.3	32	66.7	11.0
20 days prior to irradiation	48	26	54.2	22	45.8	12.0
Control	49	17	35.4	32	64.6	11.2
30 days prior to irradiation	47	10	21.3	37	78.7	9.5
Control	48	12	25.0	36	75.0	8.3

As is apparent from the data of Table 4, insertion of the pellets 10 days prior to irradiation results in a sharp increase of the radioresistance. On the twentieth day the protective effect is unquestionably retained, but only to a lesser extent. Finally, on the thirtieth day no protective action of diethystilbestrol can be detected. The picture of the action of estrogenic pellets on the survival rate of mice is revealed with sufficient clarity by the curves shown in Figure 10. The data on the basis of which these curves were plotted are shown in the addendum to the present paper (Table 9).

Observations of the course of radiation injury and of the weight dynamics exhibited by mice upon subcutaneous introduction of pellets containing diethylstilbestrol (see Table 10 of the addendum) add nothing new to that which has been stated in connection with the analysis of the foregoing analogous data.

Thus the results obtained support the possibility of a more prolonged protective action of estrogens upon their introduction into the organism in the form of pellets, rather than in the form of an oil solution.

Resolution of the problem concerning a maximum prolongation of the protective action of diethylstilbestrol is directly related to the elucidation of the question as to whether the action of this endocrine substance is weakened upon repeated administration. There are numerous researches concerned with the problem of the inurement of the organism to endocrine preparations (Kabak, 1947). However, in none of these instances has the question been studied in relation to the protective properties of estrogenic substances. Hence, we have carried out special experiments. Male mice of strain A were twice given a subcutaneous injection of 0.2 mg diethylstilbestrol dissolved in 0.2 ml of vegetable oil. The first injection was given 20 days, and the second 10 days, prior to a total Xray irradiation. The males of the control group were given injections of vegetable oil at the same intervals of time. Irradiation of the animals was carried out under the conditions usually utilized by us and at a dosage of 500r.

The first injection of diethylstilbestrol given 20 days prior to irradiation could produce no protective action (see Table 3). At the same time, if a weaker reaction of the organism to the repeated administration of the preparation were observed, the second injection of the estrogen

10 days prior to irradiation should also produce no protective effect. Thus in the case of a weaker reaction of the organism to a second administration of diethylstilbestrol, the survival rate of the animals of the two groups being compared (experimental and control) would be the same. The results of the experiments are shown in Table 5.

The data thus obtained leave no doubt that at least no rapid inurement of the organism to diethylstilbestrol takes place, and that its protective action is also retained following a repeated injection. This conclusion is well illustrated by the curves shown in Figure 11 (See also Table YI in the addendum).

Observation of the course of radiation injury show in these instances, as in all the preceding, a somewhat milder course of the disorder in the animals of the experimental groups as compared with the controls (see Table XII in the addendum).

Thus, the data obtained support the belief that the protective action can be prolonged by repeated administration to the animals of estrogenic substances.

TABLE 5

EFFECT OF REPEATED ADMINISTRATION OF DIETHYLSTILBESTROL ON THE SURVIVAL RATE OF MICE FOLLOWING TOTAL XRAY IRRADIATION

(DOSAGE 500r)

Series No	Treatment	Total number of animals	Survived		Died		Mean duration of life (days)
			Number	Percent	Number	Percent	
[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
1	Two administrations of Diethylstilbestrol, 10 and 20 days prior to irradiation	23	19	82.6	4	17.4	16.0
	Control	18	10	55.5	8	44.5	15.9
2	Two administrations of Diethylstilbestrol 10 and 20 days prior to irradiation	17	12	70.6	5	29.4	9.7
	Control	18	7	38.9	11	61.1	8.1

Protective Action of Diethylstilbestrol on Combined Use With Other Synthetic Sex Hormones

It is known that estrogens used in combination with other sex hormones produce a different action than that resulting from their single administration. As an example, we may cite the fact that on introduction into the organism of the hormone of corpus luteum, i.e., progesterone, the action of estrogenic substances is eliminated (Burrows, 1949). Of no less interest is the fact that the combined administration of estrogenic substances and other sex hormones results in the elimination of the cancerogenic action of the former. Found to be especially effective in this respect were progesterone and testosterone. Thus, it has been shown that

an administration of these hormones in combination with estrogens in the case of experimental animals precludes the occurrence of precancerous conditions as well as of neoplasms (hyperplastic and metaplastic alterations of the genital organs in rats, mice, and monkeys; fibroids in guinea pigs, leukoses and cancer of the mammary glands in mice). A synopsis including most of the work carried out on this problem is found in the monograph of Lipschutz published in 1950 (Lipschutz, 1950).

We have made it our goal to ascertain the influence of some synthetic hormones on the protective properties of diethylstilbestrol. Two hormones were used in the investigation -- progesterone and pregnenolone. The experimental procedure was essentially as follows. Ten days prior to Xray irradiation, at the same time as the administration of the estrogenic substance, the mice of the experimental group were also given another synthetic hormone. Thus, in addition to an administration of 0.05 mg diethylstilbestrol, the animal received an injection of 3 mg pregnenolone or of one mg progesterone. These substances were administered subcutaneously in the form of an oil solution. The controls of these experiments were mice which prior to irradiation were given only the oil, only diethylstilbestrol, or only the hormone of corpus luteum. The conditions and dosage of irradiation were the same as in the preceding experiments. The results of the combined effects of diethylstilbestrol and pregnenolone on the survival of mice subjected to the action of Xrays are shown in the addendum (Table XIII).

The investigation of the protective properties of diethylstilbestrol and pregnenolone was carried out in four series of experiments. The summative data show that pregnenolone as such has no protective action.

Diethystilbestrol increases, as usual, the resistance of mice to the detrimental results of irradiation, and the protective action of this preparation is not affected by the presence or the absence of pregnenolone. A statistically reliable difference is observed between the values which characterize the survival of control animals and those that characterize the survival of animals which had been given diethystilbestrol either in combination with pregnenolone, or singly.

Progesterone, which was investigated in two series of experiments, also had no protective effects as such, and the data of Table 14 of the addendum show that upon combined use of progesterone and diethylstilbestrol the protective properties of the latter are fully retained.

As in the foregoing instance, a statistically reliable difference is found between the survival rate of the control animals and that of the animals which had received diethylstilbestrol, either singly or in combination with progesterone.

Results of the investigation of the time of death among animals of the different groups are shown in the addendum (Tables XV and XVI) and are represented graphically by the curves of Figures 12 and 13. These data do not support the belief that there exists a specific effect upon the time of death of the animals resulting from the use of diethylstilbestrol in combination with the other hormones which have been tested.

Observations of the course of radiation injury in mice of the different groups reveal that administration of diethylstilbestrol, either singly or in combination with pregnenolone or progesterone, produces beneficial effects. Mice which had been given diethylstilbestrol withstood as usual the radiation injury much better. This fact is also

reflected to some extent by the data relating to the weight values. Figures 14 and 15 show the curves representing the changes in weight of the irradiated mice (those which recovered) during the 30 days of observation. The corresponding numerical data are shown in Tables XVII and XVIII of the addendum. The curves of Figure 14 show that mice which had been given diethylstilbestrol (either singly or in combination with pregnenolone) begin to gain in weight between the seventh and the twelfth day following irradiation, while the controls begin to gain in weight only between the twelfth and the sixteenth day.

Although the investigation of the combined effects of diethylstilbestrol and progesterone did not reveal any difference in the time intervals when gains in weight occur, the rate of recuperation is appreciably higher in mice which had been given both substances at the same time.

On summing up the over-all data on the combined action of diethylstilbestrol and other synthetic hormones, it sould be noted that combined introduction into animals of diethylstilbestrol and hormones, which eliminate the estrogenic and carcinogenic action of the former, has no effect whatever on the protective properties of the former.

Conclusions

1. A single prophylactic administration of synestrol, and especially of diethylstilbestrol, increases the resistance of mice to the damaging action of ionizing radiation, and manifests itself as follows:

(a) The rate of survival of the irradiated mice is increased about twofold.

(b) The course of radiation injury is less severe.

(c) The recuperation of damage induced by a single total irradiation is more rapid.

2. Within the investigated range of diethylstilbestrol dosages (from 0.025 to 0.8 mg per mouse) the effectiveness of its protective action is about the same.

3. The enhanced radioresistence of mice, induced by a single subcutaneous injection of diethylstilbestrol, manifests itself between the first and the third day following administration of the estrogenic substance and disappears between the tenth and the twelfth day. During these 10 days the protective action of the estrogen is found to be at a practically constant high level.

4. Upon subcutaneous introduction of pellets containing diethylstilbestrol, the protective action of the preparation can be prolonged 20 days.

5. By repeated administration of diethylstilbestrol it is possible to prolong its protective action.

6. Administration of pregnenolon or progesteron, the sex hormones which modify the action of estrogens, does not eliminate the protective properties of the latter.

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TABLE I

LIFE DURATION OF MICE THAT DIED FOLLOWING IRRADIATION

Treatment	Group	Total number of animals	Number of animals that died	Day of Death																	
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Administration of Synestrol	Experiment	77	33	-	-	-	3	1	2	1	5	1	4	5	3	2	1	-	2	1	-	
	Control	84	55	-	-	-	-	-	1	3	4	6	19	9	5	5	6	3	1	-	-	
Administration of Diethylstilbestrol	Experiment	89	20	-	-	-	2	-	4	1	1	1	1	1	-	2	3	1	-	1	1	
	Control	92	56	-	-	1	2	1	2	1	3	7	7	10	5	5	6	1	-	-	1	

Treatment	Group	19	20	21	22	23	24	25	26	27	28	29	30	Mean life duration (days)
Administration of Synestrol	Experiment	1	-	-	-	-	-	-	-	-	1	-	-	10.2
	Control	1	-	-	-	-	-	-	-	-	-	-	-	10.7
Administration of Diethylstilbestrol	Experiment	-	-	-	1	-	-	-	-	-	-	-	-	10.35
	Control	-	-	-	-	1	-	-	-	-	-	2	1	11.2

TABLE II

CHANGES IN WEIGHT OF THE ANIMALS IN THE EXPERIMENTAL AND THE CONTROL GROUP (THE ZERO-DAY IS THAT OF IRRADIATION)

Animal group		Number of animals and their weight	Days of Weighing									
			-10	0	+2	+7	+10	+13	+17	+21	+25	+28
Experimental group (mice having received 0.2 mg diethylstilbestrol each)	Survived	Mean weight (g)	23.0	24.5	23.0	23.0	23.5	23.9	24.6	24.8	24.8	25.0
		Index	93.8	100.0	93.8	93.8	95.9	97.6	100.6	101.2	101.2	102.1
		Number of animals	69	69	69	69	69	69	69	50	58	29
	Died	Mean weight (g)	22.3	23.4	22.0	21.3	21.2	20.1	20.2	-	-	-
		Index	95.4	100.0	94.0	91.1	90.6	85.9	86.2	-	-	-
		Number of animals	20	20	20	13	11	6	2	-	-	-
Control group	Survived	Mean weight (g)	22.2	23.8	22.6	22.3	22.2	21.7	22.4	22.6	23.0	24.2
		Index	93.3	100.0	95.0	93.7	93.3	91.2	94.1	95.0	96.6	101.8
		Number of animals	36	36	36	36	29	36	36	29	31	12

TABLE 2 (continued)

			-10	0	+2	+7	+10	+13	+17	+21	+25	+28
Control group	Died	Mean weight (g)	22.6	24.2	23.1	22.6	21.6	19.6	23.5	23.3	23.6	23.0
		Index	93.4	100.0	95.5	93.4	89.2	81.0	97.1	96.2	97.5	95.0
		Number of animals	56	56	56	49	34	11	5	3	1	2

Animal group		Number of animals and their weight	Days of Weighing									
			-10	0	+3	+6	+9	+12	+16	+20	+23	+25	+28
Experimental group	Survived	Mean weight (g)	20.6	22.9	21.4	21.1	21.4	21.5	22.2	23.2	23.1	23.6	23.4
		Index	90.0	100.0	93.5	91.2	93.5	93.9	97.0	101.3	100.9	101.3	102.3
		Number of animals	44	44	44	44	44	44	44	44	44	30	30
	Died	Mean weight (g)	20.6	22.0	20.9	20.3	18.8	18.2	20.1	23.1	22.9	18.4	-
		Index	93.6	100.0	95.0	92.4	85.4	82.7	91.4	105.0	104.1	83.6	-
		Number of animals	33	33	33	27	19	9	3	1	1	1	-
Control group	Survived	Mean weight (g)	21.2	23.3	22.1	22.3	21.8	20.9	21.8	22.3	23.4	24.8	24.7
		Index	91.0	100.0	94.8	95.7	93.5	89.7	93.5	95.6	100.3	106.4	106.0
		Number of animals	29	29	29	29	29	29	29	29	29	14	14
	Died	Mean weight (g)	21.1	23.0	21.7	21.1	19.9	17.3	14.6	-	-	-	-
		Index	91.8	100.0	94.4	91.8	86.5	77.4	63.4	-	-	-	-
		Number of animals	55	55	55	54	35	17	1	-	-	-	-

TABLE III

CHANGES OF PERIPHERAL BLOOD INDICES IN MICE FOLLOWING ADMINISTRATION OF DIETHYLSTILBESTROL

Blood Indices	Injection	Time Elapsed After Injection in hours +2	+1	+5	+10	+13	+17	+21	+28
Number of leukocytes (in thousands per 1 mm^3)	D*	6,925	7,075	7,900	7,745	6,500	7,488	7,565	7,813
	D**	7,363	10,213	11,725	9,515	9,700	10,110	8,038	8,025
Number of Erythrocytes (in millions per 1 mm^3)	D	8,283	8,207	7,350	8,432	8,260	7,995	7,565	8,435
	M	9,075	8,120	9,045	8,118	9,160	8,040	9,430	8,180
Hemoglobin (percent)	D	102	104	100	114	109	109	108	114
	M	109	105	117	111	110	110	120	106

* Diethylstilbestrol

** Oil

TABLE IV

CHANGES OF PERIPHERAL BLOOD INDICES IN IRRADIATED MICE AFTER A PRELIMINARY ADMINISTRATION OF DIETHYLSTILBESTROL

Blood Indices	Injection	Before irradiation	Time Elapsed After Irradiation In hours								
			+2	+1	+5	+9	+13	+17	+21	+25	+33	+47
Number of leukocytes (in thousands per 1 mm^3)	D*	7.745	7.088	2.265	0.583	1.317	2.917	5.250	6.783	6.425	6.050	6.525
	M**	9.515	8.544	2.417	0.234	0.790	2.367	4.542	9.317	7.050	5.600	6.610
Number of erythrocytes (in millions per 1 mm^3)	D	8.432	7.797	8.373	8.070	5.875	5,840	7.323	8.233	8.397	7.957	8.022
	M	8.188	7.829	7.303	8.297	5.012	6.287	8.190	8.810	7.968	7.660	8.003
Hemoglobin (percent)	D	114	106	116	109	96	85	102	108	122	117	115
	M	111	109	108	108	81	80	84	95	129	104	109

*Diethylstilbestrol

**Oil

TABLE V

LIFE DURATION OF MICE OF THE EXPERIMENTAL AND CONTROL GROUPS THAT DIED FOLLOWING IRRADIATION

Dose of diethylstilbestrol (in mg)	Total number of animals	Number of animals that died	Day of Death 0 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean life duration (days)
0.025	63	18	-	-	-	1	1	-	4	2	2	2	-	3	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	9.2
Control	54	35	-	-	-	1	1	1	4	2	2	12	4	4	-	2	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	9.5
0.05	89	33	-	-	-	-	1	3	5	2	6	3	2	3	-	1	4	-	1	-	-	-	-	-	1	-	1	-	-	-	-	10.3
Control	102	73	-	-	-	1	3	3	9	3	13	16	11	4	1	2	1	1	-	-	1	-	-	-	-	-	-	-	-	2	-	9.6
0.1	40	9	-	-	-	-	-	2	2	2	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	10.4
Control	40	26	-	-	-	-	1	1	1	1	2	10	3	4	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.8
0.2	89	20	-	-	-	2	-	4	1	1	1	1	1	-	2	3	1	-	1	1	-	-	-	1	-	-	-	-	-	-	-	-	10.3
Control	92	56	-	-	1	2	1	2	1	3	7	7	10	5	5	6	1	-	-	1	-	-	-	-	1	-	-	-	-	2	1		11.2
0.8	33	9	-	-	-	3	-	-	2	1	-	-	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	8.7
Control	34	25	-	-	-	-	1	1	-	1	4	7	3	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-		11.2

TABLE VI

CHANGES IN WEIGHT OF MICE FOLLOWING IRRADIATION AND ADMINISTRATION OF DIFFERENT DOSES OF DIETHYLSTILBESTROL (THE ZERO IS THAT OF IRRADIATION)

Animal groups		Number of animals and their weight	-10	0	+3	+7	+10	+14	+18	+21	+25	+29
Experimental group (mice given 0.025 mg of diethylstilbestrol	Survived	Mean weight (g)	20.8	22.2	20.0	20.7	21.5	22.1	-	22.9	23.8	24.3
		Index	93.6	100.0	90.0	93.2	97.2	99.5	-	103.2	107.3	109.5
		Number of animals	39	39	39	39	27	39	-	39	39	39
	Died	Mean weight (g)	20.8	21.5	18.7	18.4	17.7	18.4	-	-	-	-
		Index	96.7	100.0	87.0	85.6	82.3	85.6	-	-	-	-
		Number of animals	18	18	18	11	7	2	-	-	-	-
Control group	Survived	Mean weight (g)	20.7	22.3	20.5	21.2	21.1	22.0	-	22.4	23.3	23.8
		Index	93.0	100.0	92.0	95.1	94.7	98.7	-	100.5	104.4	106.8
		Number of animals	19	19	19	18	14	14	-	19	19	19
	Died	Mean weight (g)	20.6	22.2	19.6	20.1	18.6	17.1	14.0	-	-	-
		Index	92.8	100.0	88.3	90.6	83.7	77.1	63.1	-	-	-
		Number of animals	35	35	34	27	10	1	1	-	-	-
Experimental group (mice given 0.05 mg of diethylstilbestrol each)	Survived	Mean weight (g)	21.2	22.6	20.6	21.0	21.2	22.2	22.9	23.8	24.9	24.8
		Index	93.0	100.0	90.4	92.2	93.0	97.4	100.4	104.5	109.3	108.9
		Number of animals	56	56	56	56	56	56	38	45	40	56
	Died	Mean weight (g)	20.2	21.6	19.4	18.3	17.8	20.3	18.8	19.2	-	-
		Index	93.5	100.0	89.8	84.7	82.4	94.0	87.0	88.9	-	-
		Number of animals	33	33	33	24	10	4	1	2	-	-
Control group	Survived	Mean weight (g)	20.8	22.6	20.9	20.6	20.9	21.4	22.0	22.8	23.1	24.2
		Index	92.1	100.0	92.5	91.2	92.5	94.7	97.4	100.9	102.2	107.1
		Number of animals	29	29	29	29	29	29	17	22	24	29
	Died	Mean weight (g)	20.8	22.2	20.6	20.1	19.6	19.6	20.6	23.6	-	-
		Index	93.7	100.0	92.8	90.6	88.3	88.2	92.8	106.3	-	-
		Number of animals	73	73	72	56	14	4	3	2	-	-
Experimental group (mice given 0.1 mg diethylstilbestrol each)	Survived	Mean weight (g)	21.4	22.7	20.6	21.1	21.2	22.8	23.6	23.9	24.2	
		Index	94.3	100.0	90.7	93.0	93.4	100.4	104.0	105.3	106.8	
		Number of animals	31	31	31	31	31	31	31	31	31	
	Died	Mean weight (g)	23.0	24.2	22.0	22.9	22.7	24.2	24.6	23.8	-	
		Index	95.0	100.0	91.0	94.6	93.9	100.0	101.8	98.4	-	
		Number of animals	9	9	9	5	3	1	1	1	-	
Control group	Survived	Mean weight (g)	21.0	22.3	20.4	21.3	21.4	22.2	23.1	23.7	24.2	
		Index	94.2	100.0	91.5	95.6	96.0	99.6	103.7	106.4	108.6	
		Number of animals	14	14	14	14	14	14	14	14	14	
	Died	Mean weight (g)	21.2	22.5	20.0	20.4	18.1	-	-	-	-	
		Index	94.2	100.0	88.9	90.7	80.4	-	-	-	-	
		Number of animals	26	26	26	23	6	-	-	-	-	

TABLE VI (continued)

Animal group		Number of animals and their weight	Days of weighing								
			-10	0	+2	+7	+10	+13	+17	+21	+25	+28
Experimental group (mice given 0.2 mg diethylstil-bestrol each)	Survived	Mean weight (g)	23.0	24.5	23.0	23.0	23.5	23.9	24.6	24.8	24.8	25.0
		Index	93.8	100.0	93.8	93.8	95.9	97.6	100.4	101.2	101.2	102.1
		Number of animals	69	69	69	69	69	69	69	50	58	29
	Died	Mean weight (g)	22.3	23.4	22.0	21.3	21.2	20.1	20.2	-	-	-
		Index	95.4	100.0	94.0	91.1	90.6	85.9	86.2	-	-	-
		Number of animals	20	20	20	13	11	6	2	-	-	-
Control group	Survived	Mean weight(g)	22.2	23.8	22.6	22.3	22.2	21.7	22.4	22.6	23.0	24.2
		Index	93.3	100.0	95.0	93.2	93.3	91.2	94.1	95.0	96.6	101.8
		Number of animals	36	36	36	36	29	36	36	29	31	12
	Died	Mean weight (g)	22.6	24.2	23.1	22.6	21.6	19.6	22.5	23.3	23.6	23.0
		Index	93.4	100.0	95.5	93.4	89.2	81.0	97.1	96.2	97.5	95.0
		Number of animals	56	56	56	49	34	11	5	3	1	2
			-10	0	+3	+7	+10	+14	+18	+21	+27	+29
Experimental group (mice given 0.8 mg diethylstilbes-trol each)	Survived	Mean weight (g)	22.6	24.1	21.2	21.2	22.1	22.9	23.8	24.5	25.0	25.7
		Index	93.8	100.0	88.0	88.0	91.8	95.0	98.8	101.8	103.8	106.8
		Number of animals	24	24	24	24	24	24	24	24	24	24
	Died	Mean weight (g)	23.4	24.8	21.8	20.6	20.5	20.4	-	-	-	-
		Index	94.4	100.0	88.0	83.1	82.6	82.3	-	-	-	-
		Number of animals	9	9	9	6	3	2	-	-	-	-
Control group	Survived	Mean weight (g)	21.9	23.5	21.5	21.6	22.4	23.0	24.0	24.3	24.8	25.6
		Index	93.2	100.0	91.5	91.9	94.4	97.8	102.1	103.4	105.5	109.0
		Number of animals	9	9	9	9	9	9	9	9	9	9
	Died	Mean weight (g)	21.5	23.2	21.0	20.3	20.2	22.0	23.8	23.6	23.0	-
		Index	92.7	100.0	90.5	87.5	87.1	94.8	102.5	101.7	99.1	-
		Number of animals	25	25	25	23	8	2	2	2	2	-

TABLE VII

LIFE DURATION OF MICE THAT DIED FOLLOWING IRRADIATION UPON ADMINISTRATION OF DIETHYLSTILBESTROL AT DIFFERENT TIME INTERVALS PRIOR TO EXPOSURE TO XRAYS

Time of administration	Group	Total number of animals	Number of animals that died	Day of Death																												Mean duration of life (days)
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
1 day prior	Experimental	23	5	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	11.3
	Control	24	8	-	-	-	-	-	-	-	-	-	1	-	3	1	-	-	-	-	-	1	-	1	-	-	-	-	1	-	-	-	-	14.1
3 days prior	Experimental	24	6	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	1	1	-	1	-	-	-	-	-	-	-	-	1	-	-	15.7
	Control	23	16	-	-	-	-	-	-	1	3	2	4	3	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.7
5 days prior	Experimental	22	6	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	2	-	-	1	-	1	-	-	-	-	-	-	-	-	-	14.3
	Control	24	17	-	-	-	-	-	-	1	1	2	5	-	3	1	-	-	-	-	-	-	1	-	-	1	-	-	1	-	1	-	-	-	11.9
10 days prior	Experimental	89	33	-	-	-	-	1	3	5	2	6	3	2	3	-	1	4	-	1	-	-	-	-	-	-	1	-	1	-	-	-	-	-	10.3
	Control	102	73	-	-	-	1	3	3	9	3	13	18	11	4	1	2	1	1	-	-	1	-	-	-	-	-	-	-	-	-	2	-	9.6
12 days prior	Experimental	48	35	-	-	-	1	3	-	7	2	6	-	4	-	1	1	1	1	-	1	-	2	-	-	2	-	3	-	-	-	-	-	11.5
	Control	48	42	-	-	-	1	6	1	8	6	8	1	2	3	2	-	-	1	-	1	1	-	-	-	-	-	-	-	-	1	-	-	8.8
15 days prior	Experimental	22	11	-	-	-	-	-	1	2	1	1	1	1	-	-	1	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	11.0
	Control	22	13	-	-	-	-	-	2	-	2	-	1	1	-	-	4	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	11.4

TABLE VIII

CHANGES IN WEIGHT OF MICE UPON ADMINISTRATION OF DIETHYLSTILBESTROL AT DIFFERENT TIME INTERVALS PRIOR TO IRRADIATION (THE ZERO-DAY IS THAT OF IRRADIATION)

Time of administration of diethylstilbestrol	Number of animals and their weight	Days of weighing							
		0	+4	+8	+12	+16	+20	+25	+29
1 day prior to irradiation	Mean weight (g)	25.1	23.4	22.6	23.3	23.8	24.8	24.8	25.5
	Index (weight)	100.0	93.2	90.0	92.8	94.7	98.8	98.8	101.6
	Number of animals	18	18	18	18	18	17	18	18
Control	Mean weight (g)	26.2	23.8	23.6	23.3	23.8	24.8	25.1	26.3
	Index (weight)	100.0	90.8	90.1	89.0	90.8	94.6	96.9	100.4
	Number of animals	16	16	16	16	16	16	16	16

TABLE VIII (continued)

Time of administration of diethylstilbestrol	Number of animals and their weight	Days of weighing								
		-3	0	+4	+8	+12	+16	+20	+25	+29
3 days prior to irradiation	Mean weight (g)	25.2	26.4	23.9	23.9	24.9	26.0	26.2	26.3	-
	Index (weight)	95.5	100.0	90.6	90.6	94.4	98.5	99.2	99.6	-
	Number of animals	18	18	18	18	18	18	18	18	-
Control	Mean weight (g)	24.8	26.6	24.7	23.3	23.4	25.0	25.6	26.2	-
	Index (weight)	93.2	100.0	92.8	87.6	88.0	94.0	96.3	98.5	-
	Number of animals	7	7	7	7	7	7	7	7	

Time of administration of diethylstilbestrol	Number of animals and their weight	Days of weighing								
		-5	0	+4	+8	+12	+16	+21	+25	+29
5 days prior to irradiation	Mean weight (g)	22.6	24.1	21.1	21.2	21.9	22.6	23.9	23.9	24.8
	Index (weight)	93.7	100.0	87.5	87.9	90.9	93.7	99.1	99.1	102.9
	Number of animals	16	16	16	16	16	16	16	16	16
Control	Mean weight (g)	21.6	23.2	20.4	20.1	20.1	21.1	22.7	22.6	23.7
	Index (weight)	93.1	100.0	92.3	86.7	86.7	91.0	97.9	97.4	102.1
	Number of animals	7	7	7	7	7	7	7	7	7

Time of administration of diethylstilbestrol	Number of animals and their weight	Days of weighing								
		-10	0	+3	+7	+14	+18	+21	+25	+29
10 days prior to irradiation	Mean weight (g)	21.2	22.8	20.6	21.0	22.2	22.9	23.8	24.9	24.8
	Index (weight)	93.0	100.0	90.4	92.2	97.4	100.4	104.5	109.3	108.9
	Number of animals	56	56	56	56	56	38	45	40	56
Control	Mean weight (g)	20.8	22.6	20.9	20.6	21.4	22.0	22.8	23.1	24.2
	Index (weight)	92.1	100.0	92.5	91.2	94.7	97.4	100.9	102.2	107.1
	Number of animals	29	29	29	29	29	17	22	24	29

Time of administration of diethylstilbestrol	Number of animals and their weight	Days of weighing								
		-12	0	+4	+8	+12	+17	+20	+24	+29
12 days prior to irradiation	Mean weight (g)	21.4	22.5	20.2	20.0	19.8	21.1	22.2	23.1	23.8
	Index (weight)	95.1	100.0	89.8	89.0	88.1	93.9	98.6	102.8	105.9
	Number of animals	13	13	13	13	13	12	13	13	13
Control	Mean weight (g)	20.8	21.4	20.5	20.2	19.7	20.3	21.6	22.5	23.4
	Index (weight)	97.2	100.0	95.9	94.4	92.1	95.0	100.9	105.2	109.4
	Number of animals	6	6	6	6	6	6	6	6	6

Time of administration of diethylstilbestrol	Number of animals and their weight	Days of weighing					
		-15	0	+7	+14	+26	+29
15 days prior to irradiation	Mean weight (g)	23.2	25.2	24.2	24.7	25.9	26.2
	Index (weight)	92.1	100.0	96.0	98.1	102.9	104.0
	Number of animals	11	11	11	11	11	11
Control	Mean weight (g)	22.6	23.9	23.7	24.4	24.3	24.7
	Index (weight)	94.5	100.0	99.2	102.1	101.7	103.3
	Number of animals	9	9	9	9	9	9

TABLE IX

LIFE DURATION OF MICE THAT DIED FOLLOWING IRRADIATION UPON SUBCUTANEOUS INTRODUCTION OF PELLETS CONTAINING DIETHYLSTILBESTROL

Time of introduction of pellets	Group	Total number of animals	Number of animals that died	Day of Death																			
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
10 days prior to irradiation	Experimental	48	15	-	-	-	-	-	1	2	3	1	-	2	-	1	1	-	1	-	1	-	-	1
	Control	48	32	-	-	-	-	-	-	-	-	1	7	5	8	2	4	1	1	-	1	-	2	-
20 days prior to irradiation	Experimental	48	22	-	-	-	-	-	-	-	2	2	2	4	2	2	1	1	1	-	2	-	2	-
	Control	49	32	-	-	-	-	-	-	1	3	1	7	6	4	3	1	2	-	-	-	-	-	-
30 days prior to irradiation	Experimental	47	37	-	-	-	-	-	-	2	5	3	7	9	4	4	-	1	-	-	-	-	-	2
	Control	48	36	-	-	-	-	-	-	3	6	5	12	5	2	2	1	-	-	-	-	-	-	-

Time of introduction of pellets	Group	21	22	23	24	25	26	27	28	29	30	Mean life duration (days)
10 days prior to irradiation	Experimental	-	-	-	-	-	-	-	-	1	-	11.0
	Control	-	-	-	-	-	-	-	-	-	-	11.0
20 days prior to irradiation	Experimental	-	-	-	-	-	-	1	-	-	-	12.0
	Control	1	1	-	-	-	1	1	-	-	-	11.2
30 days prior to irradiation	Experimental	-	-	-	-	-	-	-	-	-	-	9.5
	Control	-	-	-	-	-	-	-	-	-	-	8.3

TABLE X

CHANGES IN WEIGHT OF MICE ON SUBCUTANEOUS INTRODUCTION OF PELLETS CONTAINING DIETHYLSTILBESTROL AT DIFFERENT TIME INTERVALS, PRIOR TO IRRADIATION (THE ZERO-DAY IS THAT OF IRRADIATION)

Time of introduction	Group	Number of animals and their weight	Days of weighing -10	0	+4	+9	+13	+20	+26	+30
10 days prior	Experimental	Mean weight (g)	21.1	21.2	19.9	20.4	21.6	22.8	23.5	24.4
		Weight index	99.5	100.0	93.8	96.2	101.8	107.5	110.9	115.1
		Number of animals	33	33	33	33	33	33	33	33
	Control	Mean weight (g)	20.7	23.2	23.0	21.9	21.4	24.0	24.6	25.9
		Weight index	89,2	100.0	99.2	94.4	92.2	103.5	106.1	110.3
		Number of animals	16	16	16	16	16	16	16	16

Time of introduction	Group	Number of animals and their weight	Days of weighing -21	-12	-3	0	+4	+10	+14	+19	+23	+28
20 days prior	Experimental	Mean weight (g)	23.2	24.0	25.4	24.1	23.7	23.6	25.1	25.8	25.8	25.5
		Weight index	96.2	99.5	105.4	100.0	98.4	97.9	104.1	107.1	107.1	105.8
		Number of animals	26	12	12	26	26	26	25	26	26	26
	Control	Mean weight (g)	22.4	23.5	25.4	24.2	24.1	22.6	23.6	24.6	24.9	25.2
		Weight index	92.6	97.3	105.0	100.0	99.6	93.0	97.6	101.8	103.0	104.1
		Number of animals	17	4	4	17	17	17	17	17	17	17

			Days of weighing -30	-16	-7	0	+5	+9	+14	+18	+ 23	+27
	Experimental	Mean weight (g)	21.2	21.4	23.4	24.4	23.8	23.8	23.9	24.9	25.3	26.0
		Weight index	86.8	87.8	95.0	100.0	97.5	97.5	98.0	102.0	103.8	106.5
		Number of animals	10	10	5	10	10	10	10	10	10	10
	Control	Mean weight (g)	20.8	23.8	24.8	25.4	24.7	24.2	24.4	25.5	25.7	26.2
		Weight index	81.9	93.7	97.7	100.0	97.2	95.3	96.1	100.3	101. 2	103.1
		Number of animals	12	12	4	12	12	12	12	12	12	12

TABLE XI

LIFE DURATION OF MICE THAT DIED FOLLOWING IRRADIATION UPON REPEATED ADMINISTRATION OF DIETHYLSTILBESTROL

Series	Treatment	Total number of animals	Number of animals that died	Day of death 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30	Mean life duration (days)
1	Two administrations of diethylstilbestrol, 10 and 20 days prior to irradiation	23	4	- - - - - - - - - - 1 - 1 - - 1 - - - - - - - - - - 1 - - - -	16.0
	Control	18	8	- - - - - - - - 1 1 1 - - 1 1 - 1 - - - - - - - 1 - - - - 1	15.9
2	Two administrations of diethylstilbestrol, 10 and 20 days prior to irradiation	17	5	- - - 1 - - 1 - - 1 - - 1 - - - 1 - - - - - - - - - - - -	9.7
	Control	18	11	- - - 2 - 2 - 2 1 - 1 - 3 - - - - - - - - - - - - - - - -	8.1

TABLE XII

CHANGES IN WEIGHT OF MICE UPON REPEATED ADMINISTRATION OF DIETHYLSTILBESTROL, 20 AND 10 DAYS PRIOR TO IRRADIATION (THE ZERO DAY IS THAT OF IRRADIATION)

Series	Group	Number of animals and their weight	Days of weighing								
			-19	-8	0	+4	+8	+12	+16	+20	+25	+29
1	Experimental	Mean weight (g)	22.0	25.4	27.0	24.4	25.2	25.4	26.2	27.0	26.3	26.9
		Weight index	81.5	94.8	100.0	90.4	93.3	94.0	97.0	100.0	97.4	99.6
		Number of animals	19	19	19	19	19	19	19	19	19	19
	Control	Mean weight (g)	21.3	23.8	24.8	23.6	23.6	22.3	23.3	23.8	24.2	23.8
		Weight index	86.0	96.0	100.0	95.1	95.1	90.0	94.0	96.0	97.6	96.0
		Number of animals	10	10	10	10	10	10	10	10	10	10

TABLE XIII

EFFECTS OF DIETHYLSTILBESTROL AND PREGNENOLON ON THE SURVIVAL RATE OF MICE FOLLOWING IRRADIATION

Series No	Administration of Pregnenolon					Administration of Diethylstilbestrol				
	Total number of animals	Animals that survived		Animals that died		Total number of animals	Animals that survived		Animals that died	
		Number	Percent	Number	Percent		Number	Percent	Number	Percent
1	23	8	34.8	15	65.2	-	-	-	-	-
2	34	9	26.4	25	73.6	12	4	33.3	8	66.7
3	18	8	44.4	10	55.6	18	17	94.4	1	5.6
4	18	7	38.9	11	61.1	17	11	64.7	6	35.3
Total	93	32	34.4±4.8	61	65.6±4.8	47	32	68.1±6.8	15	31.9±6.8

TABLE XIII (end)

Series No	Administration of diethylstilbestrol and pregnenolon					Control				
	Total number of animals	Animals that survived		Animals that died		Total number of animals	Animals tha survived		Animals that died	
		Number	Percent	Number	Percent		Number	Percent	Number	Percent
1	-	-	-	-	-	23	3	13.0	20	87.0
2	-	-	-	-	-	29	2	6.9	27	93.1
3	18	12	66.7	6	33.3	18	10	55.6	8	44.4
4	18	12	66.7	6	33.3	17	7	41.2	10	58.8
Total	36	24	66.7±7.8	12	33.3±7.8	87	22	25.3±4.7	65	74.7±4.7

TABLE XIV

EFFECTS OF DIETHYLSTILBESTROL AND PROGESTERONE ON THE SURVIVAL RATE OF MICE FOLLOWING IRRADIATION

Series No	Administration of Progesterone					Administration of diethylstilbestrol				
	Total number of animals	Animals that survived		Animals that died		Total number of animals	Animals that survived		Animals that died	
		Number	Percent	Number	Percent		Number	Percent	Number	Percent
1	18	17	94.4	1	5.6	24	22	91.7	2	8.3
2	18	8	44.5	10	55.5	17	15	87.2	2	11.8
Total	36	25	69.4±7.7	11	30.6±7.7	41	37	90.3±4.6	4	9.7±4.6

TABLE XIV (end)

Series No	Administration of Diethylstilbestrol and Progesterone					Control				
	Total number of animals	Animals that survived		Animals that died		Total number of animals	Animals that survived		Animals that died	
		Number	Percent	Number	Percent		Number	Percent	Number	Percent
1	16	15	93.7	1	6.3	12	6	50.0	6	50.0
2	24	18	75.0	6	25.0	24	13	54.2	11	45.8
Total	40	33	82.5±6.0	7	17.5±6.0	36	19	52.8±8.3	17	47.2±8.3

TABLE XV

LIFE DURATION OF MICE OF THE EXPERIMENTAL AND CONTROL GROUPS WHICH DIED FOLLOWING IRRADIATION

Nature of treatment	Total number of animals	Number of animals that died	Day of death																													Mean life duration (days)
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Administration of Pregnenolon	93	61	-	-	-	1	1	-	2	4	9	16	12	5	6	2	-	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-		10.2
Administration of Diethylstilbestrol	47	15	-	-	-	-	-	2	1	-	5	1	1	1	1	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		9.8
Administration of Pregnenolon and Diethylstilbestrol	36	12	-	-	-	-	-	3	1	3	1	1	1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-		8.7
Control	87	65	-	-	-	-	1	5	6	4	9	15	12	7	3	3	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-		9.5

TABLE XVI

Nature of treatment	Total number of animals	Number of animals that died	Day of death																													Mean life duration (days)
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Administration of Progesterone	36	11	-	-	-	-	-	1	1	-	2	2	-	2	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		9.7
Administration of Diethystilbestrol	41	4	-	-	-	-	-	-	-	-	-	1	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		9.5

TABLE XVI (continued)

			01	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Administration of Diethylstilbestrol and Progesterone	40	7	-	-	-	-	-	-	1	-	3	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	11.3
Control	36	17	-	-	-	-	-	-	-	2	7	3	2	-	1	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	10.2

TABLE XVII

CHANGE IN WEIGHT OF MICE FOLLOWING IRRADIATION ON COMBINED ADMINISTRATION OF PREGNENOLON AND DIETHYLSTILBESTROL (ZERO-DAY IS THAT OF IRRADIATION

Animal group		Number of animals and their weight	Days of weighing								
			-10	0	+3	+7	+12	+16	+20	+25	+28
Given 3 mg of Pregnenolon	Survived	Mean weight (g)	20.6	22.8	21.8	21.2	20.6	21.2	22.3	23.4	23.6
		Index	90.4	100.0	95.6	93.0	90.4	93.0	97.8	102.7	103.5
		Number of animals	32	32	32	32	31	32	32	32	31
	Died	Mean weight (g)	20.4	22.4	21.5	20.3	18.5	18.3	23.7	-	-
		Index	91.0	100.0	96.0	90.7	82.6	81.7	105.4	-	-
		Number of animals	61	61	61	56	13	3	1	-	-
Given 0.05 mg of diethyl-stilbestrol	Survived	Mean weight (g)	20.8	22.5	21.0	20.7	20.9	21.4	22.4	23.4	24.0
		Index	92.5	100.0	93.3	92.0	93.0	95.1	99.6	104.0	106.7
		Number of animals	32	32	32	32	32	32	32	32	32
	Died	Mean weight (g)	19.9	21.4	19.9	17.9	16.7	-	-	-	-
		Index	93.0	100.0	93.0	83.6	78.0	-	-	-	-
		Number of animals	15	15	15	12	4	-	-	-	-
Given 3mg of pregnenolon and 0.05 mg of die-thylstilbestrol	Survived	Mean weight (g)	20.3	22.9	21.2	21.4	21.4	22.3	23.2	24.1	24.3
		Index	88.7	100.0	92.6	93.5	93.5	97.5	101.2	105.2	106.1
		Number of animals	24	24	24	24	24	24	23	24	24
	Died	Mean weight (g)	20.3	22.9	20.8	19.3	17.0	-	-	-	-
		Index	88.7	100.0	90.8	84.3	74.3	-	-	-	-
		Number of animals	12	11	12	6	2	-	-	-	-
Control	Survived	Mean weight (g)	20.6	22.8	21.8	21.8	21.4	21.7	22.8	23.7	24.6
		Index	90.4	100.0	95.6	95.6	93.9	95.2	100.0	104.0	108.0
		Number of animals	22	22	22	22	22	22	22	22	22
	Died	Mean weight (g)	20.2	21.8	20.8	19.8	17.6	17.4	-	-	-
		Index	92.7	100.0	95.4	90.8	80.7	79.8	-	-	-
		Number of animals	65	65	65	51	12	1	-	-	-

TABLE XVIII

CHANGES IN WEIGHT OF MICE FOLLOWING IRRADIATION UPON COMBINED ADMINISTRATION OF PROGESTERON AND DIETHYLSTILBESTROL (ZERO-DAY IS THAT OF IRRADIATION

Animal group		Number of animals and their weight	Days of weighing								
			-10	0	+3	+8	+12	+16	+20	+24	+29
Given 1 mg of Progesteron	Survived	Mean weight (g)	20.3	22.2	21.3	20.2	20.8	21.4	22.9	23.4	24.0
		Index	91.5	100.0	95.0	91.0	93.6	96.4	103.2	105.5	108.0
		Number of animals	25	25	25	25	25	25	25	25	25
	Died	Mean weight (g)	22.0	23.9	22.2	22.0	21.0	-	-	-	-
		Index	92.1	100.0	92.9	92.1	87.9	-	-	-	-
		Number of animals	11	11	11	5	3	-	-	-	-
Given 0.05 mg of diethylstil-bestrol	Survived	Mean weight (g)	21.2	22.8	21.3	20.9	21.6	21.4	23.4	23.5	24.4
		Index	93.0	100.0	93.5	91.8	94.7	93.9	102.7	103.1	107.1
		Number of animals	37	37	37	36	31	37	37	37	33
	Died	Mean weight (g)	22.3	24.0	21.8	20.3	-	-	-	-	-
		Index	93.0	100.0	90.9	83.4	-	-	-	-	-
		Number of animals	4	4	4	3	-	-	-	-	-
Given 1 mg of Progesteron and 0.05 mg of Diethylstilbes-trol	Survived	Mean weight (g)	20.0	22.6	21.3	20.5	21.9	22.6	24.2	24.4	25.5
		Index	89.5	100.0	94.3	90.7	97.0	100.0	107.1	108.0	113.0
		Number of animals	33	33	33	33	33	33	33	33	33
	Died	Mean weight (g)	20.3	22.0	20.0	18.0	18.9	21.2	-	-	-
		Index	92.4	100.0	91.0	81.9	85.9	96.4	-	-	-
		Number of animals	7	7	7	3	2	1	-	-	-
Control	Survived	Mean weight (g)	21.2	23.2	21.6	21.1	21.2	22.2	23.6	23.8	25.4
		Index	91.4	100.0	93.1	91.0	91.4	95.6	101.7	102.5	109.4
		Number of animals	19	19	19	19	19	19	19	19	19
	Died	Mean weight (g)	21.2	22.9	21.2	18.9	17.9	16.3	18.9	-	-
		Index	92.6	100.0	92.6	82.5	78.2	71.2	82.5	-	-
		Number of animals	17	17	17	10	3	1	1	-	-

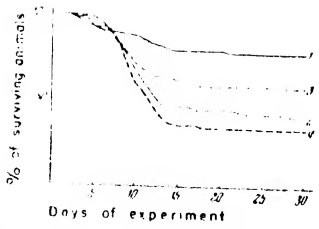

Figure 3. [illegible] duration of mice follo[illegible] ir[illegible]diation upon administration of synestrol and diethylstilbestrol.
1, diethylstilbestrol; 2, control of diethylstilbestrol; 3, synestrol; 4, control of synestrol.

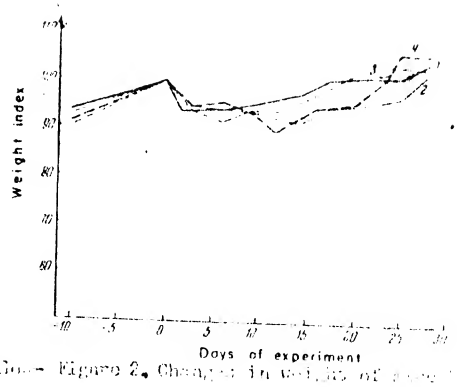

Figure 4. Changes in weight of [illegible] following irradiation [illegible] administration of synestrol [illegible] diethylstilbestrol (the [illegible] day is that of irradiation)
1, diethylstilbestrol; 2, [illegible] trol of diethylstilbestrol; [illegible] synestrol; 4, control of [illegible]

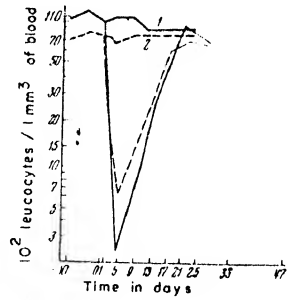

Figure 5. Changes in the number of blood leukocytes in the irradiated and nonirradiated mice on administration of diethylstilbestrol.
1, oil; 2, diethylstilbestrol; 3, oil + irradiation 600 r; 4, diethylstilbestrol + irradiation [illegible]

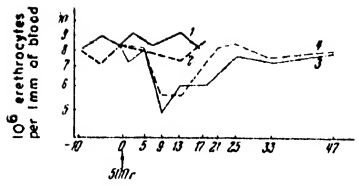

Figure 4. Changes in the number of blood erythrocytes in irradiated and nonirradiated mice upon administration of diethylstilbestrol.

1, oil; 2, diethylstilbestrol; 3, oil + irradiation 500 r; 4, diethylstilbestrol + irradiation 500 r.

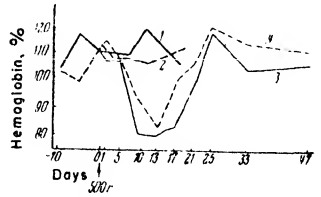

Figure 5. Hemoglobin content of the blood of irradiated and non-irradiated mice on administration diethylstilbestrol.

1, oil; 2, diethylstilbestrol; 3, oil + irradiation 500 r; 4, diethystilbestrol + irradiation 500 r.

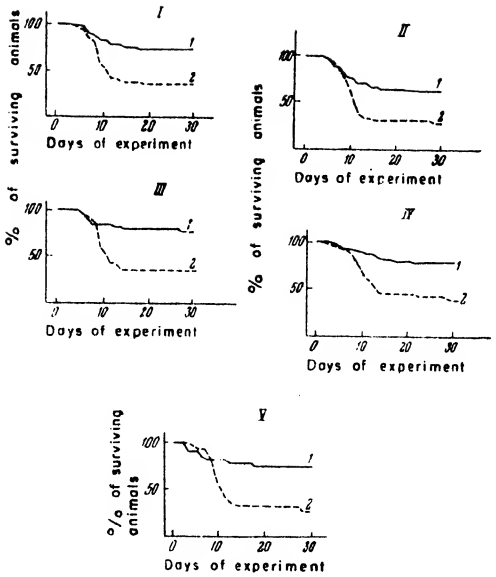

Figure 6. Life duration of mice following irradiation.

I - upon administration of 0.025 mg diethylstilbestrol;

II - upon administration of 0.05 mg diethylstilbestrol;

III - upon administration of 0.1 mg diethylstilbestrol;

IV - upon administration of 0.2 mg diethylstilbestrol;

V - upon administration of 0.8 mg diethylstilbestrol.

1, experiment; 2, control.

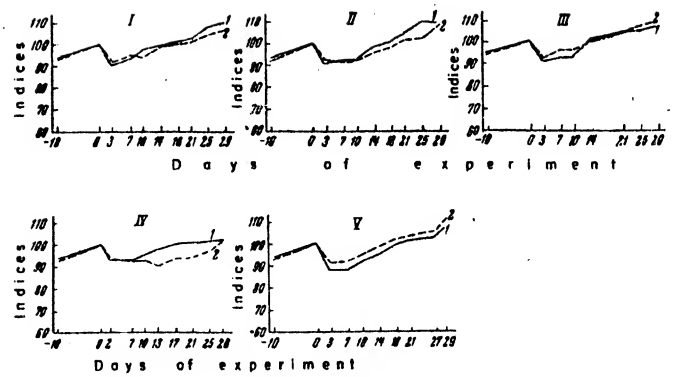

Figure 7. Changes in weight of mice following irradiation.

I - upon administration of 0.025 mg diethylstilbestrol;

II - upon administration of 0.05 mg diethylstilbestrol;

III - upon administration of 0.1 mg diethylstilbestrol;

IV - upon administration of 0.2 mg diethylstilbestrol;

V - upon administration of 0.8 mg diethylstilbestrol.

1, experiment; 2, control.

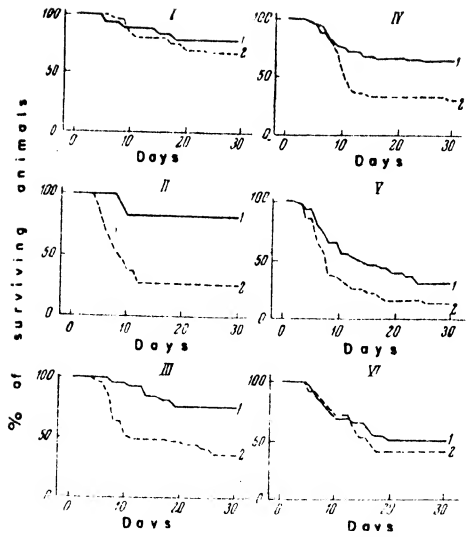

Figure 8. Life duration of mice following irradiation.

I - upon administration of diethylstilbestrol one day prior to X-ray irradiation; II - upon administration of diethylstilbestrol 3 days prior to X-ray irradiation; III - upon administration of diethylstilbestrol 5 days prior to X-ray irradiation; IV - upon administration of diethylstilbestrol 10 days prior to X-ray irradiation; V - upon administration of diethylstilbestrol 12 days prior to X-ray irradiation; VI - upon administration of diethylstilbestrol 15 days prior to X-ray irradiation.

1, experiment; 2, control.

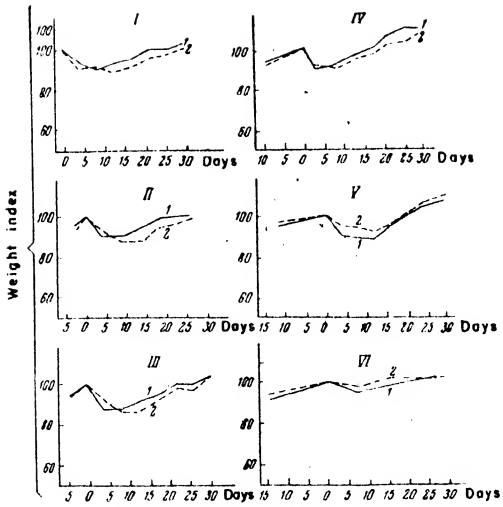

Figure 9. Changes in weight of mice following irradiation.

I - upon administration of diethylstilbestrol one day prior to X-ray irradiation; II - upon administration of diethylstilbestrol 3 days prior to X-ray irradiation; III - upon administration of diethylstilbestrol 5 days prior to X-ray irradiation; IV - upon administration of diethylstilbestrol 10 days prior to X-ray irradiation; V - upon administration of diethylstilbestrol 12 days prior to X-ray irradiation; VI - upon administration of diethylstilbestrol 15 days prior to X-ray irradiation.

1, experiment; 2, control.

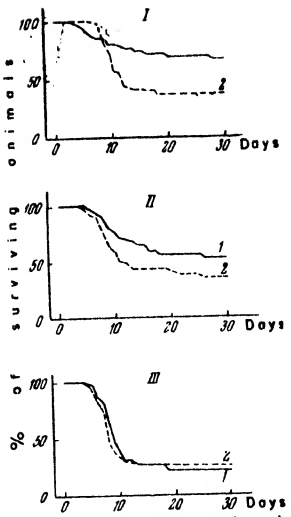

Figure 10. Life duration of mice following irradiation.

I - upon introduction of pellets contaning diethylstilbestrol 10 days prior to X-ray irradiation; II - 20 days prior to X-ray irradiation; III - 30 days prior to X-ray irradiation.

1, experiment; 2, control.

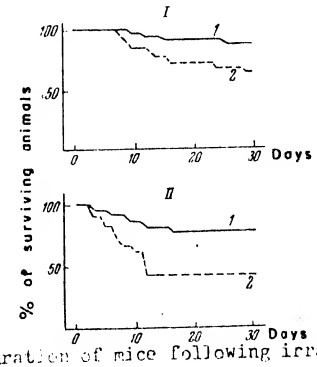

Figure 11. Life duration of mice following irradiation upon repeated administration of diethylstilbestrol (series I and II).

1, experiment; 2, control.

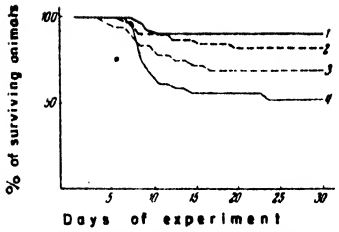

Figure 12. Life duration of mice following irradiation upon adminstration of diethylstilbestrol in conjunction with progesterone. 1, diethylstilbestrol; 2, diethylstilbestrol + progesterone; 3, progesterone; 4, control.

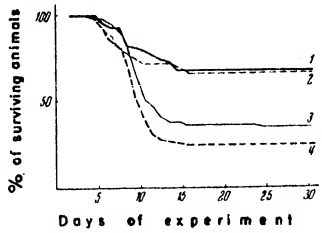

Figure 13. Life duration of mice following irradiation upon adminstration of diethylstilbestrol in conjunction with pregnenolon. 1, diethylstilbestrol; 2, diethylstilbestrol + pregnenolon; 3, pregnenolon; 4, control.

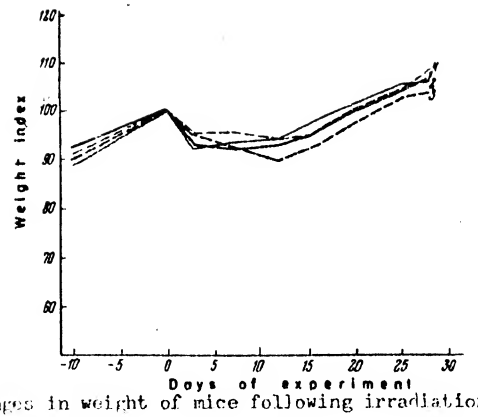

Figure 14. Changes in weight of mice following irradiation upon administration of diethylstilbestrol in conjunction with pregnenolon.

1, diethylstilbestrol; 2, diethylstilbestrol + pregnenolon; 3, pregnenolon; 4, control.

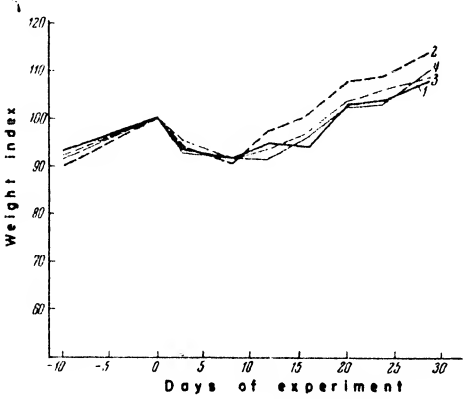

Figure 15. Changes in weight of mice following irradiation upon administration of diethylstilbestrol in conjunction with progesterone.

1, diethylstilbestrol; 2, diethylstilbestrol + progesterone; 3, progesterone; 4, control.

THE ROLE OF THE PHYSIOLOGICAL STATE OF THE ORGANISM ON UTILIZATION OF PROTECTIVE REMEDIES AGAINST THE DAMAGING ACTION OF PENETRATING RADIATIONS

N. I. Shapiro,
A. M. Kuzin
and N. I. Nuzhdin

Among the contributions concerned with the biological action of penetrating radiations, there are a sufficiently large number of investigations which indicate a correlation between the radiosensitivity of organisms and their physiological state. This correlation has been repeatedly demonstrated in the case of plant as well as of animal forms (Dugger, 1936). In some researches the role of the physiological state has been revealed upon the study of individual variations in the radiosensitivity of the irradiated objects, in others on observation of organisms at different stages of embryonic or postembryonic development, and finally in still other researches by means of alterations of the state of animals or plants due to the effects of environment. The facts which show a correlation between radiosensitivity and the physiological state of the organism are of exceptional importance primarily as substantiation of the possibility of an active intervention in the reaction of the organism to radiation exposure. In other words, the facts which show a correlation between the radiosensitivity of an organism and its physiological state constitute a certain experimental substantiation of the possibility of finding substances which protect the organism against the damaging action of penetrating radiations. From the ascertaining of these facts also follows still another most important consequence, the significance of which is at the present time clearly underestimated. Since the physiological state of the organism affects its radiosensitivity,

it is quite natural that this state must also affect the action of many protective agents inasmuch as it is unquestionable that many of these substances exercise their effects by altering the physiological state of the animal or plant being protected.

All of the foregoing apparently indicates the necessity, in searching for and undertaking the study of substances which protect biological objects from the damaging action of radiations, of taking into consideration the physiological state of the organism. It is entirely probable that effectiveness of many protective remedies will depend upon the physiological characteristics of the animals undergoing exposure. Yet in the overwhelming majority of investigations conducted in this field, the physiological state of the experimental animals is not taken into account. Such an abiological approach constitutes perhaps one of the most characteristic features of the current researches which are being conducted abroad on the study of the protective action of various chemical substances.

The abiologism is further heightened by the nature and dosages of chemical substances which are being tested in this work. At the present time intensive studies are being carried out on the protective action of such strong poisons as, for example, the cyanides (Herve and Bacq, 1949), or of huge doses of less harmful substances such as, for instance, cysteine, reaching the limit of tolerance (Smith, Patt, Tyree, and Straube, 1950). Thus, the researchers operate with means of such potency in the presence of which it is not always, by far, the case that the physiological state of the experimental animals can be of any significance. The action of substances of this kind has no relationship whatever with a utilization or furtherance of the protective mechanisms of the organism itself and constitutes rather something extraneous to the irradiated object.

Without denying the importance of the work on uncovering protective means, the mechanism of the action of which is not associated with the protective reactions inherent to the organism itself, we believe that a limitation of the scope of researches to such strongly acting means would be erroneous. There is no doubt that means which enhance the protective characteristics of the organism itself should be of no lesser, but possibly of even greater, significance.

In this connection, we consider it of special importance not only to substantiate theoretically the necessity of taking into account the physiological state of the organism, but also to show experimentally the correlation between this factor and the protective action of any given preparation. We have selected diethylstilbestrol which, according to previously derived data, was found to exercise a good protective action upon the irradiation of mice (see preceeding communication of the present symposium (Shapiro, Nuzhdin, Kuzin, 1955)).

Although the mechanism of the protective action of diethylstilbestrol as yet remains uncertain, there is no doubt that it is associated with some specific effects of estrogenic hormones on individual organs and tissues of the animals.

According to previously conducted experiments diethylstilbestrol increases approximately twofold the rate of survival in mice exposed to lethal dosages of Xrays (Shapiro, Nuzhdin, Kuzin, 1955). Table 1 shows the data on the survival rate of mice following a single Xray irradiation with a dosage of 500r. The conditions of irradiation (in this instance, as well as in all subsequent experiments) were as follows. Voltage 160 kv; current intensity 5 ma; filters 0.75 mm Al +0.5 mm cu; focal distance 40 cm; dosage

intensity 15.3r/min. The irradiation technique has been described in detail in one of our papers (Shapiro and Nuzhdin, 1955).

Ten days prior to the Xray irradiation, each mouse was given a subcutaneous injection of 0.05 mg diethylstilbestrol dissolved in 0.2 ml of refined vegetable oil. The control animals received only the oil. Sexually mature mice of strain A, not yet having propagated, aged 2-3 months, were used in this experimental series. The initial weight of the animals varied between 20 and 24 g. Observations of the experimental mice were continued (in this instance and in the other experiments) for 30 days.

The data of Table 1 leave no doubt concerning the protective action of diethylstilbestrol which manifests itself to an equal extent in female and male animals. Thus, the results obtained appear to indicate that the action of this preparation can hardly depend extensively upon the physiological state of the organism, since even such a great difference as exists between males and females does not alter its effectiveness. Nevertheless, we have made an attempt to determine the conditions which change the results of the action of the preparation under study. Since we are dealing with an estrogenic substance, it could be expected that its efficacy should depend upon the general hormonal level of the experimental animals and the associated therewith of specific changes in the functional state of the different organs. Considering that the factor which alters drastically the physiological state of the entire organism and in particular its hormonal conditions is the begetting of offspring in females, a comparison was made of the protective action of diethylstilbestrol in virgin females and those which had previously propagated. A preliminary determination was made of the radiosensitivity of these two types of animals, irrespectively of any supplementary influence.

TABLE 1

EFFECTS OF A PROPHYLACTIC ADMINISTRATION OF DIETHYLSTILBESTROL ON SURVIVAL RATE OF FEMALE AND MALE MICE OF STRAIN A FOLLOWING TOTAL XRAY IRRADIATION (DOSAGE 500r)

Object	Treatment	Total number of animals	Survived		Died		Mean life duration (days)
			Number	Percent	Number	Percent	
Females	Diethylstilbestrol	45	29	64.4±7.1	16	35.6±7.1	12.9
	Control	48	14	29.2±6.4	34	70.8±6.4	9.8
Males	Diethylstilbestrol	89	55	62.9±5.1	34	37.1±5.1	10.3
	Control	102	29	28.5±4.4	73	71.5±4.4	9.6

TABLE 2

RADIOSENSITIVITY OF VIRGIN AND OF PARENT FEMALE MICE OF STRAIN A (DOSAGE OF EXPOSURE, 500r)

Object	Total number of animals	Survived		Died		$M_{dif} \pm m_{dif}$ (%%)	Mean life duration (days)
		Number	Percent	Number	Percent		
Virgin females	48	14	29.2±6.6	34	70.8±6.6	29.1±10.5	9.8
Bred females	36	21	58.3±8.2	15	41.7±8.2		13.1

The results of experiments on the survival of virgin female mice and those which had propagated (twice), of strain A, following Xray irradiation (dosage 500r) are shown in Table 2, from which it is apparent that the radiosensitivity of virgin females is greater than that of the females which had propagated. There are reasons for assuming that the greater physiological stability of females which had given birth is not selective, as concerns the harmful consequences of Xray irradiation, but can be revealed also as concerns other unfavorable conditions.

The testing of the protective action of diethylstilbestrol in virgin females and those which had propagated was effected in the following manner. Ten days prior to Xray irradiation (dosage, 500r) each female of the experimental group was given a subcutaneous injection of 0.05 mg diethylstilbestrol dissolved in 0.2 ml of vegetable oil. The control animals received the same amount of oil. Table 3 shows the results of this experiment.

The tabulated data show that the protective action of diethylstilbestrol, which is so clearly manifested in the case of virgin females, is practically absent in the case of the females which had previously propagated. Thus, the relatively high resistance of the females which had previously propagated cannot be enhanced to any appreciable extent by an administration of diethylstilbestrol. From the data presented, it is also apparent than an administration of diethylstilbestrol to virgin females increases their radioresistance only up to the level which is characteristic of the females which had previously propagated. In this connection it is also important to bear in mind the fact that the protective action of diethylstilbestrol in males is quantitatively similar to that in virgin females (See Table 1), and that the level of radioresistance in males after they have received diethylstilbestrol is also proximate to the level found in females which had previously propagated.

TABLE 3

EFFECTS OF DIETHYLSTILBESTROL (0.05 mg) ON THE SURVIVAL RATE OF VIRGIN MICE AND THOSE WHICH HAD PROPAGATED, OF STRAIN A, FOLLOWING TOTAL XRAY IRRADIATION

(DOSAGE 500r)

Object	Treatment	Total number of animals	Survived		Died		$M_{dif} \pm m_{dif}$ (%%)	Mean life duration (days)
			Number	Percent	Number	Percent		
Virgin female	Diethylstilbestrol	45	29	64.4±7.1	16	35.6±7.1	35.2±9.7	12.9
	Control	48	14	29.2±6.6	34	70.8±6.6		9.8
Females which had propagated	Diethylstilbestrol	35	23	65.7±8.0	12	34.3±8.0	7.4±11.5	12.6
	Control	36	21	58.3±8.2	15	41.7±8.2		13.3

On the basis of the comparison of all the data obtained it is natural to assume that the difference in radiosensitivity between virgin females and those which had propagated, on the one hand, and the females which had propagated and males, on the other, must be attributed to a difference in their hormonal (estrogenic) level. This assumption can also be confirmed by facts well known in experimental oncology (Grinshteyn, 1951). The development of the cancer of mammary glands in mice requires on the one hand a cancer virus (the so-called lactary factor), and on the other the action of estrogen hormone. Only the simultaneous presence of both these components determines the development of neoplasms. In this connection, in mice of strain A which are characterized by the presence of the lactary factor, the breast tumors occur only in females which have propagated. In order to induce the occurrence of tumors in males or females which have not propagated, of this strain of mice, it is necessary to administer additional estrogenic substances.

Thus, these data also indicate that in females of strain A which have propagated the level of estrogenic hormones is much higher, not only as compared with the males but also as compared with the females which had not propagated. Since, after receiving estrogen, the males and virgin females become similar in radiosensitivity to the females which had propagated, it must be assumed that in this instance increased radiostability under the action of an estrogenic hormone is governed to a certain extent by the so-called law of "all or nothing." In other words, the attainment of a definite level of resistance requires a definite minimum of the hormone; additions thereof in excess of this amount are practically useless. This standpoint is supported by the results of our previous experiments on ascertaining the protective action of different dosages of diethylstilbestrol. Ten days

prior to Xray irradiation (dosage 500r), male mice of strain A, 2 to 3 months of age, were given subcutaneous injections of different amounts of diethylstilbestrol dissolved in vegetable oil. The data thus obtained are shown in Table 4.

On examining the data of this table it is necessary to bear in mind that, since in our investigations a fairly large variation was observed in the death rate of the irradiated animals from one experiment to another, controls were used concurrently with all the experimental series. To facilitate a comparison of the protective action of various doses of diethylstilbestrol, an index was computed, which we have designated as the survival index and which constitutes the ratio of the percent of animals that survived in the experimental group to the percent of animals which survived in the control group.

The data listed in Table 4 show that a tenfold or more increase of the diethylstilbestrol dose does not result in corresponding increase of the resistance of the mice. A somewhat higher survival rate of mice following the administration of 0.8 mg diethylstilbestrol is not statistically reliable. Thus, on the basis of these data we also arrive at the conclusion that the protective action of diethylstilbestrol is governed in practice by the so-called law of "all or nothing."

All the above cited data leave no doubt that the protective action of diethylstilbestrol depends on the physiological state of the organism subjected to irradiation, and as we have shown, in particular on its hormonal conditions. The question thus arises as to whether the presence or absence of the protective action of diethylstilbestrol is associated only with the hormonal conditions.

TABLE 4

EFFECTS OF DIFFERENT DOSES OF DIETHYLSTILBESTROL ON THE SURVIVAL RATE OF MICE OF STRAIN A FOLLOWING IRRADIATION (DOSAGE 500r)

Dose of diethylstilbestrol (in mg)	Experiment					Control					Survival Index
	Total number of animals	Survived		Died		Total number of animals	Survived		Died		
		Number	Percent	Number	Percent		Number	Percent	Number	Percent	
[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
0.025	63	45	71.4±5.6	18	28.6±5.6	54	19	35.2±6.5	35	64.8±6.5	2.03
0.05	89	56	62.9±5.0	33	37.1±5.0	102	29	28.5±4.4	73	71.5±4.4	2.20
0.1	40	31	77.5±6.6	9	22.5±6.6	40	14	35.0±7.4	26	65.0±7.4	2.22
0.2	89	69	77.5±4.4	20	22.5±4.4	92	36	39.1±5.1	56	60.9±5.1	1.98
0.8	33	24	72.7±7.7	9	27.3±7.7	34	9	26.5±7.6	25	73.5±7.6	2.74

A certain answer to this question is provided by the results of our experiments concerned with the determination of the effects of diethylstilbestrol on mice of strain C_{57} (black). The mice C_{57} (black) are extensively used in experimental ontology. They are characterized (in contrast to strain A) by the absence of the so-called lactic factor which induces the occurrence of cancer of the mammary glands. In addition, a number of researches have established numerous physiological and biochemical differences between these two strains of mice (Grinshteyn, 1951), of special interest to us is the investigation which has shown that screening of the spleen during total Xray irradiation sharply increases the survival rate of the animals of strain A and, to a much lesser extent, that of strain C_{57} (black) (Kaplan and Janice, 1952).

Studies of the strain C_{57} (black) were initiated with a determination of its radiosensitivity and a comparison with the radiosensitivity of mice of strain A. In the investigation, use was made of mice, 2 to 3 months old, which had not yet propagated, weighing from 20 to 24 g. The results relating to the survival rate of mice of both strains during the 30 days after a total Xray irradiation (dosage 500r) are shown in Table 5.

TABLE 5

RADIOSENSITIVITY OF MICE OF STRAIN A AND C_{57} (BLACK) (DOSAGE OF EXPOSURE, 500r)

Strain	Sex	Total number of animals	Survived		Died		$M_{dif} \pm m_{dif}$ (%%)	Mean life duration (days)
			Number	Percent	Number	Percent		
A	Females	48	14	29.2±6.6	34	70.8±6.6	20.8±9.7	9.8
C_{57} (black)		48	24	50.0±7.2	24	50.0±7.2		15.8
A	Males	47	15	31.9±6.8	32	68.1±6.8	24.2±9.4	9.6
C_{57} (black)		57	32	56.1±6.6	25	43.9±6.3		9.0
A	Males and	95	29	30.5±4.7	66	69.5±4.7	22.8±6.8	9.7
C_{57} (black)	females	105	56	53.3±4.9	49	46.7±4.9		12.4

As is readily apparent from the data listed in Table 5, the radiosensitivity of male and female mice of strain C_{57} (black) is lower than that of the males and females of strain A.

Tests of the protective action of diethylstilbestrol on mice of strain C_{57} (black) were started with a dose of 0.05 mg. Dissolved in vegetable oil, the preparation was administered subcutaneously 10 days prior to irradiation. The data of Table 6 show that administration of this amount of diethylstilbestrol had only a fairly slight protective action. At the same time, upon administration of a larger amount of diethylstilbestrol (0.2 mg), its effects were clearly manifested.

Thus, diethylstilbestrol protects mice of strain C_{57} (black) from the damaging action of Xray irradiation upon utilization of somewhat greater doses than in the case in regard to animals of strain A. Evidently the physiological characteristics of mice of strain C_{57} (black) are such that, in spite of their higher radioresistance, the protective action of diethylstilbestrol is manifest in them to a lesser extent. It is possible that the reverse relationship observed between the natural radioresistance of mice and their sensitivity to diethylstilbestrol is far from being fortuitous.

Adverting to the question concerning the causes of the different effects of the estrogen on mice of strains A and C_{57} (black), it can only be assumed that these causes are different from those which we have encountered in studying the action of the preparation on virgin females and those which have propagated.

TABLE 6

EFFECTS OF DIETHYLSTILBESTROL ON THE SURVIVAL RATE OF MALE MICE OF STRAINS A AND C_{57} (BLACK)

FOLLOWING TOTAL XRAY IRRADIATION (500r)

Strain	Dose of diethyl-stilbestrol	Total number of animals	Survived		Died		$M_{dif} \pm m_{dif}$ (%%)	Mean life duration (days)
			Number	Percent	Number	Percent		
A	0.05	89	56	62.9±5.0	33	37.1±5.0	34.4±6.7	10.3
	Control	102	29	28.5±4.4	73	71.5±4.4		9.6
	0.2	89	69	77.5±4.4	20	22.5±4.4	38.4±6.7	10.3
	Control	92	36	39.1±5.1	56	60.9±5.1		11.2
	0.05	37	27	73.0±7.3	10	27.0±7.3	11.2±9.6	12.1
	Control	34	21	61.8±6.3	13	38.2±6.3		10.5
C_{57} (black)	0.2	22	18	81.8±8.2	4	18.2±8.2	34.0±13.3	15.7
	Control	23	11	47.8±10.4	12	52.2±10.4		7.4

Whereas in the latter case, the difference can be attributed to the hormonal (estrogenic) levels of the two groups of animals referred to, this cannot be said as concerns the mice of strains A and C_{57} (black). Even on assuming that the estrogenic level of females of the C_{57} (black) strain is higher than that of females of the A, we cannot possibly consider that the estrogenic level of males of the C_{57} (black) strain is higher than that of virgin females of the A. Thus, the specific features of the reaction of animals of strain C_{57} (black), as compared with the animals of strain A, to the administration of diethylstilbestrol must be attributed to some physiological differences which are not directly related to the hormonal level of the animals. In other words, the means whereby the protective action of diethylstilbestrol is effected evidently depend on the physiological characteristics of the organism in the sufficiently wide meaning of this term.

In connection with the establishment of the facts which indicate that diethylstilbestrol increases the radioresistance of the organism within the limits of the existing physiological norms, it is important to discuss the question concerning the maximum dosage of Xray irradiation at which the estrogen retains its protective properties. It was natural to assume that, if the preparation does not increase the radioresistance of the animals beyond that which is encountered under normal conditions, it should also not protect the organism in cases of exposure to absolutely lethal dosages. As was shown in another paper (Shapiro and Nuzhdin, 1955) the minimum absolutely lethal dosage of Xrays in the case of male mice of strain A is 700r. Three small series of experiments were carried out with a dosage of 700r, which yielded fully conclusive results. In the first series 18 male mice of strain A, which, 10 days prior to the exposure, had been given subcutaneously 0.05 mg of diethylstilbestrol each, were irradiated. At the same time

five control animals were irradiated. All the mice -- the experimental as well as the controls -- died (the mean life duration of the mice was 11.3 days in the experimental group and 6.5 days in the control group). In the second series the dose of diethylstilbestrol was increased to 0.4 mg. The irradiation was carried out on 12 males which had received diethylstilbestrol and 12 control males. As in the first series, all the animals died -- and at approximately the same intervals of time (the mean life duration of the mice was respectively 6.2 and 5.5 days). Finally in the third series use was made of the most resistent group of animals, the females of strain A which had previously propagated. Ten days prior to irradiation, 12 females were administered 0.2 mg of diethylstilbestrol, and 12 females were irradiated without any preliminary treatment. In this instance, as well, all the animals died (there was a mean life duration of 9.0 days in the control group, and of 8.2 days in the experimental group).

Thus, on summarizing the results of these experiments, it can be stated that diethylstilbestrol does not increase the resistance of the animals to such extent that they are capable of withstanding the deleterious effects of irradiation in the presence of an absolutely lethal dosage. The experiments have shown that dosages of Xray irradiation at which diethylstilbestrol exercises its protective action do not exceed the limits of those at which, even under normal conditions, i.e., without an administration of the hormonal preparation, a small portion of the mice survive. It is possible that diethylstilbestrol, while protecting some organs of the animals, has no effect on other organs, and therefore death occurs as a result of the damage to the latter. Thus, all the data cited in the present paper indicate that diethylstilbestrol is a preparation the action of which is associated with the protective mechanisms of the organism itself; therefrom follow all its positive and negative properties.

The materials which we have considered, relating to the role of the physiological state of the organism upon the use of diethylstilbestrol, evoke a number of general questions which are significance, in principle, as concerns the search for and the study of substances which protect the organism from the damaging action of penetrating radiations. First of all, it is desirable to provide an answer to the question as to what significance may be attributed to the study of substances the action of which is directly associated with the protective mechanisms which are inherent to the organism itself. Evidently in such instances we will encounter, as a rule, an increase of the radioresistance of the organism only within the limits of lethal, but not absolutely lethal, dosages of exposure. In addition, the efficacy of such preparation may depend to a large extent upon the physiological state of the objects subjected to irradiation.

The above-stated, seemingly unfavorable circumstances notwithstanding, we believe that the study of substances intended to enhance the protective properties of the organism itself is of great interest not only theoretically, but also practically. It is unquestionable that in the course of investigations on this kind of preparations there may be ascertained the concrete physiological nature of the so-called radiosensitivity of the organisms. Up to the present time, the widespread use of this concept in radiobiology notwithstanding, it is to a considerable extent devoid of a concrete physiological meaning. The importance of a cognizance of the nature of radiosensitivity is further emphasized by the fact that this constitutes a concomitant cognizance of the concrete means of the action of radiation upon the organism. Of no lesser importance may also be the study of substances of this kind in the practice of safeguarding the organism against the damaging action of penetrating radiations. It is quite natural that one

must learn how to protect the organism from radiation damage not only on exposure to absolutely lethal dosages but also to ordinarily lethal dosages. At the same time we believe that protection of the organism on exposure to absolutely lethal dosages also requires the discovery of preparations which enhance the natural radioresistance of the organism. Radiation injury in animals, arising on their exposure to large dosages of radiation, constitutes in practice a result of damages to all organs and tissues. Hence, both preventive methods and treatment procedures in regard to this disorder will be of a complex, composite nature. There are no reasons for hoping that in this instance it will be possible to find some kind of single, universal therapeutic remedy. Thus, the composite control of radiation injuries requires the utilization of the most diversified preparations. Among these not the least position, by far, will be allocated to those preparations which enhance the protective properties of the organism itself.

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CONCERNING THE ROLE OF DAMAGE TO HEMATOPOIETIC ORGANS IN THE COURSE OF RADIATION REACTION

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INTRODUCTION

One of the most characteristic features of the damaging action of penetrating radiations inflicted to mammals under conditions of a total irradiation in the disruption of the structures and functions of all the systems of the organs and tissues of the animal. However, it is well known that, in spite of such total damage, not all the tissues of the organism, by far, are equally radiosensitive. In other words, different organs and tissues of the irradiated animal by far do not become damaged to the same extent. The urgent necessity arises of ascertaining the role of the damage to individual organs in the course of the radiation reaction. This analysis, in addition to its theoretical significance in determining the nature of the

radiation reaction in mammals, may be also of a more direct practical value in connection with the necessity of developing effective procedures for the treatment of radiation damages.

At the present time there are two principal methods for ascertaining the role of the damage to individual organs and tissues during the course of the general radiation reaction in mammals. The first method is a local irradiation of the animals (and correspondingly a screening of individual organs on general irradiation), and the second is the implantation of individual organs and tissues to animals having been exposed to radiation. By mutually supplementing each other, both these methods permit the deriving of the correct solution of the postulated problem.

In studying the role of the damage to different organs in the course of radiation reaction, the researchers devoted special attention to ascertaining the significance of damage to the hemopoietic system. This concern is due on the one hand to the exceptionally important role of the blood and hemopoietic organs in the vital activities of the animals, and on the other to their high radiosensitivity. As was shown by numerous investigations, the hemopoietic tissue is one of the most radiosensitive tissues of the organism (Yegorov and Bochkarev, 1950).

Leaving out of consideration the numerous morphological investigations concerned with the study of the changes in the blood and hemopoietic organs under the influence of ionizing radiations (see the synopsis of this work in the book of Yegorov and Bochkarev "Krovotvoreniye i ioniziruyushchaya radiatsiya" [Hematopoiesis and Ionizing Radiations], 1950), we will mention the contributions in which a study was made of the role of the hematopoietic organs in the radiation reaction by a screening of these organs during the

general irradiation of the animals or by the implantation of hemopoietic organs of nonirradiated animals to the irradiated animals (Jacobson, Marks, Robson, Gaston, and Zirkle, 1949; Mandart, Lambert, and Maisin, 1952a, 1952b; Barnes and Loutit, 1953; Laugendorff, Koch, and Sauer, 1954).

The overall result of these investigations, the objects of which were in most instances small rodents (mice, rats), was a conclusion as to the very important role of damage to the hemopoietic organs in the course of the radiation reaction in mammals. In spite of the unquestionably conclusive nature of this deduction, it is necessary to note that a number of features involved in the damage to the hemopoietic organs and their correlations with the most important aspects of the course of the radiation reaction have remained unelucidated.

One must take into account the procedural difficulties which are encountered in the study of the problems under consideration. It is precisely these difficulties that are responsible to a considerable extent for the great variations in the experimental results of different researchers. The latter applies to the data secured on screening the hemopoietic organs of the animals during irradiation (Kaplan and Janice, 1952), as well as on the transplantations of these organs (Barnes and Loutit, 1953; Langendorff, Koch, and Sauer, 1954). Variations in the results were found to be so pronounced that they have been the subject of a special research in which the author has studied the influence of the hereditary features of sex and age of the experimental animals on the curative effects of transplanted spleen (Cole and Ellis, 1953).

In carrhing out the study of the regularities of the radiation reaction in mammals, and in particular, while determining the significance of the damage to specific organs as concerns the course of this reaction,

we also had to investigate the role of the hemopoietic organs. The present paper is devoted to the description of the results of experiments carried out in this direction.

Material and Method

The material consisted of male and female mice of strain A, aged from 2 to 3 months. The mice were subjected to Xray irradiation under the following conditions: voltage 160 kv; current intensity 5 ma; filters: 0.5 cu + 0.75 Al; focal distance 40 cm; dosage 18.3 r/min. Observations of the irradiated mice were continued for 30 days following the exposure. Determination was made of the time of death of the animals, changes in their weight, and the general course of the radiation reaction. In a number of cases observations were conducted on the peripheral white blood components. Weighing of the experimental mice was done every fourth day, and blood samples were taken once every 5 days.

The investigation consisted of three parts. In the first, a comparison was made of the course of radiation reaction in the totally irradiated males and females with screened spleen or bone marrow (rear extremities). Screening was done with lead plates 3 mm thick, in the manner shown in Figure 1.

Withdrawal of the spleen was effected without narcosis of the animals operated upon. The mouse was fastened supinely to the board. At the left upper portion of the abdomen an incision about 1 cm in length was made. Through this incision the spleen was withdrawn, pulled aside, and placed in a sterile gauze bandage moistened with physiological solution and held in a small lead cell. During this operation the spleen is not damaged and its connection with the organism is not disrupted, in particular all of the

blood vessels remaining intact. This operation was performed on the animals of the experimental series (on screening of the spleen), as well as on the controls (without screening of the spleen). On completion of the irradiation, the spleen was put back into the abdominal cavity and the incision was sutured. Screening of the bone marrow was effected by the covering of one or both rear extremities.

In the second part of the investigation the transplantation of the spleen from nonirradiated mice to the irradiated was carried out. In these cases the irradiation was carried out, using special containers which could hold 12 animals. In so doing, the focal distance was reduced to 20 cm. The dosage was 7.4 r/min. Total dose of exposure was 500r.

We have tested a number of procedures of spleen transplantation. In some cases, a spleen taken from an adult mouse of the same strain was transplanted during the first hour following irradiation to male and female mice of strain A. In transplantation the vessels of the spleen of the donor were tied up, and the transplanted organ was sewed to the epiploon of the recipient. In the animals of the control series pieces of the epiploon of the donor mice were sewed on. In other cases, from one to three spleens of 8-22 day old mice were transplanted to the males during the first hour following irradiation. The operation technique is the same as in the preceding cases. The use of these procedures of transplanting the spleen did not yield the expected result, viz., a higher survival rate of the irradiated animals. In this connection, a new procedure was tested. The principal variant of the experiments, the results of which are given in the present paper, was carried out in the following manner. As recipients, female mice of strain A which had given birth to a litter were used, while the donors were the offspring, at the age of 1-5 days, of each female. To each female

there were transplanted four to five spleens. By this procedure we ensured the greatest consanguinity between the tissues of donor and recipient which, as is known, provides the most favorable conditions for the adaption as well as the survival of the transplanted organs and tissues. By this transplantation procedure we excluded to the maximally possible extent those difficulties in the adaption of the transplanted organ which are connected with tissue incompatibility. Thus, the procedure utilized makes it possible to form an objective opinion on the basis of relatively limited experimental material concerning the prospects of the effected transplantation of any given organs or tissues.

In our experiments the spleens for transplantation were prepared half an hour before irradiation. The removed spleens were kept in a sterile Ringer solution. During the first hour following irradiation the transplantation of the spleens to mice of the experimental group was carried out. For this purpose the females were fastened to a special board. No narcotic was used during the operation. At the left side of the abdomen a small incision of the skin was made, and the abdominal cavity was exposed. The spleens being transplanted were inserted into the abdominal cavity in such manner as to place them in the region of the epiploon (recessus lienalis) which adjoins the spleen of the recipient mouse. The vessels of the transplanted spleens were not tied, and the spleens were not attached to any organs or the epiploon. After the insertion of the spleens the incision was sutured. The control group of animals consisted of female mice which had given birth at the same time as the animals of the experimental series. The control animals were operated on in the same manner, but without the insertion of spleens. Finally, in the third part of the investigation a study was made of the effects of an intravenous administration of homologous

bone marrow upon the course of the radiation reaction in mice. Male mice of strain A, aged 2-3 months, were used both as donors and as recipients in these experiments. The conditions and technique of exposure to Xrays were the same as in the studies of the effects of spleen transplantation upon the survival rate of the irradiated animals.

During the first hour following the Xray irradiation, the mice of the experimental group were given an intravenous tail injection of 0.6 ml of a bone marrow suspension in a buffer solution. The injected volume of the suspension contained the bone marrow isolated from the two femurs of a nonirradiated animal. Mice of the control group were injected with a corresponding amount of the buffer solution. The buffer solution contained in one lit of distilled water NaCl (6.8g), KCl (0.4g), $CaCl_2$ (0.2g), $MgSO_4$ (0.1g), NaH_2PO_4 (0.125g), $NaHCO_3$ (2.2g), and glucose (0.1g). The pH of the solution was 7.4. The suspension of bone marrow was prepared in the following manner. Femurs thoroughly cleaned to remove muscles and fasciae were placed (in pairs from each donor) in vessels containing sterile Ringer solution. To remove the marrow, the upper end of the femur was cut off, and the needle of a syringe was inserted in the opening. The bone was only slightly incised at the lower tip, and this end was immersed in a small vessel holding 0.6 ml of the buffer solution. By means of the syringe, this solution was drawn 2 or 3 times through the bone, thereby removing the marrow therefrom. The bone was then removed from the needle, and the same procedure was repeated with the second femur of the donor. As a result, the concentration of the thus prepared suspension corresponded to the bone marrow of the two femurs of the donor.

Effect of the Screening of Hematopoietic Organs Upon the Course of Radiation Reaction in Mice

To study the role of the damage to hematopoietic organs in the course of the radiation reactions in the animals, three fundamental kinds of experiments were first carried out. In one (control) experiment a total Xray irradiation of the animals was carried out. In the second, a total irradiation with screening of the spleen. Finally, in the third a total irradiation was combined with a screening of the rear extremities of the animals. In the different variants of the experiments different doses of Xray exposure were used. The purpose of this portion of the work was to determine the changes in the nature of the reaction of the animals under conditions of a complete preservation of different hematopoietic organs. Securing of these data was not only of interest to use per se but was also of great importance in the carrying out of subsequent experiments.

The results of a total irradiation of the mice with Xrays and of the irradiation under conditions of a screening of spleen and bone marrow are shown in the synoptic Table 1.

(See Table 1 on Page 97)

This table shows data relating to the survival of the animals and the mean duration of life of the mice that died.

Examination of the tabulated data shows that screening of any of the studied hematopoietic organs increases sharply the survival of the animals. In most cases, in spite of the relatively small number of animals investigated, the results relating to experimental and control series show statistically reliable differences.

TABLE 1

SURVIVAL OF MICE FOLLOWING XRAY IRRADIATION CARRIED OUT UNDER CONDITION OF A SCREENING OF THE HEMATOPOIETIC ORGANS

Experimental series	Dose (r)	Group	Total number of animals	Survived		Died		Mean duration of life (days)
				Number	Percent	Number	Percent	
Screening of spleen	500	Experiment	27	19	70.4±8.8	8	29.6±8.8	14.6
		Control	24	10	41.6±10.1	14	58.4±10.1	11.4
	600	Experiment	17	11	64.7±11.6	6	35.3±11.6	9.5
		Control	17	2	11.7±7.8	15	88.3±7.8	8.6
	800	Experiment	21	7	33.4±10.3	14	66.6±10.3	6.0
		Control	20	0	0.0	20	100.0	4.6
Screening of bone marrow	600	Screening of one extremity	34	24	70.6±7.9	10	29.4±7.9	11.0
		Screening of two extremities	34	30	88.2±5.5	4	11.8±5.5	19.2
		Control	34	6	17.6±6.5	28	82.4±6.5	9.6
	700	Screening of one extremity	23	7	30.4±9.6	16	69.6±9.6	6.3
		Control	24	0	0.0	24	100	5.6

As was to be expected, a more complete protection of bone marrow (screening of two extremities) is more effective than a partial (screening of one extremity), although a direct proportionality is not observed in these instances.

On comparing the protective effect resulting from screening of the spleen and bone marrow, it is noted that screening of the spleen yields very similar results to those obtained by a partial screening of bone marrow and is somewhat less effective than protection of both rear extremities. Worthy of attention are the results of the screening of the spleen at different doses of exposure. In these cases the greater the irradiation dosage (within the range of ordinarily lethal but not absolutely lethal dosages), the greater the relative protective action of the screening. Thus, the survival of the animals following an irradiation dosage of 500r under the conditions of a screening of the spleen is only 1.7 times greater than that of the controls; whereas, with a dosage of 600r, it is more than 5 times greater.

Evidently the death of the animals following relatively small irradiation dosages is associated to a lesser extent with a damage to hematopoietic organs. It should be noted that this conclusion is in good agreement with literature data and also with our own unpublished data showing a relatively slight damage to hematopoietic organs following chronic exposure to small doses of radiation (Zirkle, 1954).

From the data shown in Table 1, still another unquestionable conclusion follows: damage to hematopoietic organs plays an important, possibly a decisive, part in the death of the irradiated animals upon application of the minimal absolutely lethal dose or even a somewhat higher dose). This is evidenced by the relative increase in stature of the

role of the screening of hematopoietic organs in the survival of the animals upon increase of the irradiation dosage, as well as by the high rate of survival of the screened animals (30-33%) following exposure either to a minimal absolutely lethal dose (700r) or a higher dosage (800r). In other words, it may be assumed that determination of the level of exposure (irradiation dosage) at which a 100% death rate of the irradiated animals occurs is connected primarily with a damage to the hematopoietic organs. On comparing our results with the literature data concerning the role of the damage to individual systems of organs in the radiation reaction following different doses of Xray exposure, the following assumption can be made. Upon application of relatively small dosages, death of the animals results from a general weakening of the irradiated organism which involves no predominant damage of any one specific system of organs or tissues. Death of the animals following exposure to a dose of the order of $LD_{50-100/30}$ is due primarily to a disruption of hematopoietic functions. Finally, upon application of considerably higher dosages of the order of $LD_{100/3}$, the determinant role in the course of the radiation reaction appertains to a damage of the digestive system and above all of the intestines (Quastler, Lanzl, Keller, and Osbone, 1951). The beneficial effect of a screening of the hematopoietic organs on the course of the radiation reaction can be ascertained not only from the final result, viz., the survival of the animals, but also from the course of this reaction. A relatively milder course of the radiation reaction is evidenced primarily by the behavior and appearance of the irradiated animals. This is also indicated, in particular, by the mean values of life duration of the mice that died, which are always higher in the experimental than in the control series. Finally, this is made still more apparent upon analysis of the data which characterize the dynamics, in time, of the death of irradiated

animals. Figure 2 shows a graph which represents the time of death of irradiated animals in regard to cases involving screening of the spleen and those without such screening (the data on the basis of which this and the following graph are plotted are shown in the addendum of the present paper. Figure 3 shows curves of the same nature which reveal the effects of a screening of the bone marrow.

Herein, as well as in the case of a safeguard of the spleen, the shape of the animal-survival curves relating to the experimental series differs sharply from those relating to the control series. This difference is made especially evident upon comparison of the curves, showing the survival of animals which were subjected to irradiation as well as screening of both rear extremities, with the curves relating to the controls. In this instance death occurrences among animals of the experimental series begin at a time when such occurrences completely ceased among those of the control series.

A good index of the beneficial effect of the screening of hematopietic organs upon the course of the radiation reaction of the animals is provided by the dynamics of their change in weight. Figure 4 shows the curves of weight changes in surviving mice during the 30 days following irradiation. The irradiation was carried out with and without screening of the spleen.

To facilitate a comparison of the curves, the weight of the animals is expressed in the form of indices (ratio of mean weight of the mice at the time of weighing to the mean weight at the beginning of the experiment, which is taken as being equal to 100).

Analysis of the curves shown in the graph reveals a lesser decrease in weight among animals irradiated under the conditions of a safeguard of the spleen, as compared with those which were not so protected. This is especially manifest on examination of the data relating to irradiation with a dosage of 500r. Most characteristic in the weight changes of the animals of all the experimental groups, in comparison with the controls, is the acceleration of the recuperative processes. It is also of interest to note that the changes in weight of the animals irradiated with a dosage of 800r while their spleen was screened, are about equivalent to the changes in weight observed in animals following a total irradiation with dosages of 500-600r.

Figure 5 shows the curves of the changes in weight of mice which have been subjected to irradiation with and without screening of the extremities. No particular differences are found in the shape of the weight variation curves relating to animals irradiated with screening of one and both extremities. Both these curves are characterized by a relatively rapid drop followed by a sharp increase which contrasts with the curve relating to the controls.

Examination of the shape of the curves which characterize changes in weight of the experimental animals and of the controls, permits, on the whole, arriving at the following general conclusion. Screening of hemato-poietic organs at the time of Xray irradiation manifests itself not so much by a decrease of the primary overall damage to the organism as by the rapidity and intensity of the recuperative processes.

Effect of Spleen Implantations Upon the Course of Radiation Reaction in Mice

The beneficial action of spleen screening upon the course of radiation reaction in mice naturally suggested the possibility of attaining the same

effect not by screening of this organ but by means of its transplantation from nonirradiated to irradiated animals. Experiments of this nature, as was mentioned hereinbefore, have been carried out by a number of researchers and have yielded positive results (Jacobson, Simmons, Marks, and Eldredge, 1951; Barnes and Loutit, 1953, et al.). At the same time, up to now the mechanism of the positive influence of the implantations has not been determined. The fate of the transplanted spleens has not been followed, and an effective transplantation procedure has not even been developed, i.e., a procedure which would make certain a positive action of the transplants upon the course of the radiation reaction. One of the characteristic features of the work carried out in this direction is the very great variability of the results. All this made necessary further study of the effects of a transplantation of the spleen of nonirradiated animals upon the course of the radiation reaction.

As was stated hereinbefore, the principal variant of our experiments on determination of the effects of spleen implantation upon the course of the radiation reaction consisted in a transplantation of the spleens of the offspring to their mothers. To ascertain the fate of transplanted spleens, the mice of the experimental series were dissected 2 to 2 1/2 months thereafter. Thus, it was found that, in addition to their own spleen, about 60% of the animals also had 3 to 4 spleens which had undergone adaption. The adapted spleens are smaller in size than normal (but are considerably larger than their size at the time of transplantation) and have the appearance of normally functioning organs, situated most frequently at the inner wall of the abdominal cavity. A ramified network of blood vessels extends to the transplanted spleens. The picture observed during one of the dissections is shown in the photograph of Figure 6.

A histological analysis of the implanted spleens has been carried out. Fixation of the material (pieces of the tissues of the implanted and the natural spleen) was conducted in a Zenker solution. The preparations were stained by hematoxylin with eosin (for an examination of the general microscopic structure of the organs), according to the Mallory method (to reveal the extent of development of connective elements), and with azure-eosin (for a differential staining of the cellular blood elements).

Mention should be made of the great similarity in the microscopic structure of the natural and implanted spleens. The adapted spleens, as well as the natural, are enclosed in a connective-tissue capsule. Both kinds of spleen show normally developed white and red pulps; there is present a reticular syncytium in the meshes of which are found free cells including erythrocytes, megakaryocytes, very numerous lymphocytes, a small number of neutrophiles and eosinophiles. The enumerated cellular forms are found at most diverse stages of development. The implanted and natural spleens are permeated with blood vessels and in the Malpighian bodies there is invariably found an eccentrically disposed central artery. Staining with azure-eosin clearly reveals the presence in the Malpighian bodies of more lightly stained areas, the centers of cell proliferation.

A detailed comparison of the adopted and natural spleens also ascertains certain differences in their microscopical structures. As was stated above, both kinds of spleen have a well developed connective tissue capsule; nevertheless, while in the natural spleen numerous fairly coarse and thick trabeculae extend from the capsule into the depth of the tissues, in the implanted spleens the trabeculae are few, very thin, and delicate, and it is only at the places where the vessels enter the spleen that the

trabecular are of a fully developed form (see Figures 7 and 8).

In the implanted spleens the Malpighian bodies are more numerous than in the natural spleen. Moreover, in the adapted spleens the Malpighian bodies have more definite contours (see Figures 9 and 10). In the Malpighian bodies of both the natural and the implanted spleens there are observed a large number of cells undergoing division; nevertheless, in the implanted spleens the mitoses proceed with greater intensity (see Figure 11).

Within different areas of the adopted spleens there can be observed a considerable amount (greater than in the natural spleen) of eosinophiles (up to 12 to 14 within the field of vision) and megakaryocytes (up to 8 to 9 within the field of vision) at different stages of development. Some of these cells are in process of undergoing division (see Figures 12 and 13).

Many of the differences noted between the implanted and the natural spleens are probably due to age differences between the organs being compared. As was stated in the part of the paper relating to the procedures, the spleens for implantation were taken from mice one to 5 days of age, whereas the recipients were adult mice aged 2 1/2 to 3 months.

All the above-presented observations permit reaching the conclusion that in the irradiated mice the adopted, as well as the natural, spleens are the sites of active hematopoiesis.

TABLE 2

SURVIVAL OF THE ANIMALS FOLLOWING IRRADIATION. EXPERIMENT: IRRADIATED MICE WITH TRANSPLANTED SPLEENS, CONTROL: IRRADIATED MICE, HAVING UNDERGONE NO SPLEEN IMPLANTATION

Series	Total number of animals	Survived		Died		Mean duration of life (days)
		Number	Percent	Number	Percent	
Experiment	71	53	74.6±5.1	18	25.4±5.1	9.5
Control	63	27	42.8±6.2	36	57.2±6.2	10.8

We note in addition that the histological analysis data constitute still another confirmation of the previously made assumption concerning the great importance, in relation to the results of the investigation, of the procedure utilized in the experiments involving a study of the influence of spleen transplantation on the course of radiation reaction. Thus, in particular, the data obtained by us are of an opposite nature in comparison with those recently published by Langendorff, et al. The procedure used in the latter research did not ensure an adaption of the transplanted spleens (Langendorff, Koch, and Sauer, 1954).

The results which we have obtained indicate a greater effectiveness of the procedure of transplanting organs and tissues from newborn animals to their mothers. This method may be of value also in other experimental work concerned with the study of the mechanism involved in the effects produced by transplantation of various organs on the course of the radiation reaction.

Table 2 shows data which characterize the survival of irradiated females with and without implanted spleens. As is apparent from the tabulated data, the survival of the animals of the experimental series exceeds almost twofold that of the controls. The differences observed are statistically reliable. Conspicuous is the fact that the mean life duration of the mice which died in the experimental series is less than that of the controls. Usually, under the conditions of a safeguarding, the animals live longer than the controls. Later on we will give further consideration to this somewhat unexpected fact.

The beneficial effect of spleen implantation is most clearly manifested upon examination of the survival curves shown in Figure 14.

The survival curve of the animals of the experimental series is characterized by a more elevated level as compared with the control series. We note in this connection that the death rate of the two groups being compared is almost the same up to the eighth day. Thereafter, the death instances among animals with implanted spleens occur at a somewhat slower rate, and cease almost entirely after the thirteenth day. Death instances among mice of the control series subside only on the seventeenth day, but isolated instances of death occurrence are encountered up to the last (thirtieth) day of observation. This nature of the death rate among experimental and control animals is precisely the reason which brings about the fact that mean life duration of the mice which died in the experimental group is found to be shorter than in the case of the controls.

The observations of animals with implanted spleens have shown that a transplantation of the spleen renders milder a number of symptoms of the radiation reaction. The general appearance of the experimental mice is better than that of the controls, as are the weight indices and the data which characterize the dynamics of changes in the number of leukocytes found in the peripheral blood. Figure 15 shows the curves which characterize the changes in the number of leukocytes in the animals of the groups being compared over the 30-day period of observation.

The group of experimental animals appears to be subdivided into two subgroups, one of which includes the mice in which (according to the results of the subsequent dissections) the spleens have become adapted, while the other comprises those animals in which an adaption of the spleens has not taken place,

Accordingly, the experimental group of animals is represented in the graph, in addition to the summative curve (curve 1), by still two other curves. In all cases a decrease in the number of leukocytes occurs during the 5 days following irradiation. The greatest difference between experiment and control manifests itself in the recuperatory process. In the mice of both experimental groups the recuperatory process takes place more intensively (especially in mice with adopted spleens) than in the control animals.

On examination of the data relating to the changes in the weight of the mice, represented in the form of curves in Figure 16, we also perceive that in the animals with adapted spleens the decrease in weight is somewhat less than in the controls, and, what is of special interest, that in the former the recuperative processes are effected much more rapidly and to a fuller extent. Here, as in the case involving the leukocytes, we are in a position to ascertain that the adaption of spleens manifests itself essentially by an accelerated progress of recuperation. Mice in which the spleens have not become adapted constitute a separate instance. Their weight after definite intervals of time following irradiation is found to be even below that of the controls. The cause of this is obscure. If this instance is omitted from consideration, all the above-presented data indicate that implantation of the spleen, carried out by the above-described procedure, has a most beneficial effect on the irradiated animals. This manifests itself by a greater survival and a less pronounced nature of the radiation reaction. The latter is objectively evidenced by the dynamics of changes in the number of leukocytes and weight among the irradiated animals.

Effects of Intravenous Administration of Bone Marrow on the Course of Radiation Reaction in Mice

The beneficial action of the screening of bone marrow (as well as of the screening of the spleen) on the general irradiation of the animals suggests the possibility of its administration to irradiated animals with the view of rendering milder the radiation reaction in these animals. The study of this question by means of experiments on mice is of unquestionable interest, primarily for the reason that it is precisely the bone marrow that constitutes the principal hematopoietic organ in highly developed mammals. Attempts to administer bone marrow derived from nonirradiated mice to irradiated mice have been reported in the literature. In some instances an unquestionably positive effect was observed, with increasing survival of the irradiated animals (Rekers, Coulter, and Warren, 1950; Lorenz, Congdon, and Uphoff, 1952); in other instances no such effects were noted (Talbot and Pinson, 1951). The conflicting results thus obtained and also the undetermined nature of the mechanism of the action of bone-marrow administration in those experiments which yielded positive results have led us to undertake the study of this question. Therein are presented the preliminary data which we have obtained on the effects of an intravenous injection of bow marrow upon the course of radiation reaction in mice.

Table 3 shows the data which characterize the survival of irradiated animals following administration of bone marrow (experiment) and without such administration (control).

The higher percentage of survival among animals of the experimental series, as compared with the controls, leaves no doubt as to the beneficial action of an administration of bone marrow upon the course of the radiation reaction in mice. The same is also indicated by the greater life duration

of the animals which died.

Figure 17 shows curves which illustrate the death rate of the experimental animals and that of the controls, following irradiation. In these cases, as in those involving implantation of spleen, the difference in death rate values among experimental and control animals becomes apparent only after the eighth day.

TABLE 3

SURVIVAL OF ANIMALS FOLLOWING IRRADIATION. EXPERIMENT: MICE TO WHICH BONE MARROW WAS ADMINISTERED INTRAVENOUSLY AFTER IRRADIATION. CONTROL: IRRADIATED MICE TO WHICH NO BONE MARROW WAS ADMINISTERED

Series	Total number of animals	Survived		Died		Mean duration of life (days)
		Number	Percent	Number	Percent	
Experiment	104	55	52.9±4.5	49	37.1±4.5	10.3
Control	104	37	35.5±4.7	67	64.5±4.7	9.8

Starting from this point of time, death occurrences among control mice take place at a considerably more rapid rate than among the experimental animals, and last up to about the twentieth day. Thereafter, only isolated instances of death occur among animals of both series.

As in the preceding experiments on screening of hematopoietic organs and implantation of spleen, an investigation was also made of the other objective indices of the course of the radiation reaction. These indices include first of all the amount of leukocytes in the peripheral blood of irradiated animals. Figure 18 shows the curves which characterize the

changes in the number of leukocytes in mice injected with bone marrow after irradiation, and in the controls.

As is apparent from these curves leukopenia occurs at the same time in the animals of both groups being compared, and it reaches an identical level. At the same time the recuperatory process takes place much more intensely in mice which have been given an injection of bone marrow. Thus, the number of leukocytes in the blood of the control animals reaches only the twentieth day the level which it had attained on the fifteenth day in the mice of the experimental series.

Not less indicative is the manifestation of the beneficial action of bone-marrow injections upon examination of data relating to changes in the weight of the irradiated animals. Figure 19 shows the pertinent data.

In mice of the experimental group, maximum weight decrease is noted on the fourth day after irradiation, which is followed by a gradual increase. By the sixteenth day the animals reach their initial weight. Mice of the control group reveal quite a different picture. In these cases, decrease in weight continues up to the twelfth day following irradiation. The initial weight is reached only on the twenty-fourth day. Thus, all the data presented in this section indicate the beneficial action of an intravenous injection of bone marrow on the radiation reaction in mice.

Discussion

It appears of unquestionable interest to compare the data characterizing the course of radiation reaction in mice which have been exposed to

radiation under conditions of screening of hematopoietic organs, with the course of reaction in animals which have had a spleen implantation or an injection of bone marrow after the irradiation. Of no lesser interest is a comparison of the data obtained on the study of the radiation reaction in animals with protected or implanted spleen, with analogous data relating to animals with screened bone marrow or injected with bone marrow.

Notwithstanding the fact that these experiments were carried out not at the same time, their comparison is fully permissible. Their comparability is determined by the similarity of conditions both physical (Xray irradiation) and biological (the keeping of the animals) maintained on conducting these experiments. The presence of such similarity in conditions is evidenced in particular by the values which characterize the survival of the animals of the different control series.

In comparing the data obtained, it is necessary first of all to point out the great similarity in the effectiveness of all the tested protection methods. For a comparison of the efficacy of the different protection methods, it is better to utilize not the percent of survival of the animals in any given series of experiments, but rather the so-called survival index, i.e., the ratio of percent of surviving animals in the experiment to the percent of surviving animals in the control. The survival index on exposure to a dose of 500r, with screening of the spleen, is 1.7, on implantation of the spleen it is also 1.7; and, finally, with injection of bone marrow it is 1.5 (see data shown in Tables 1, 2, and 3). Such coinciding values of the survival index can hardly be considered a fortuitous occurrence.

An especially important feature which is common to all the tested protection forms is in our opinion the predominant influence of the protection upon the course of the recuperatory processes and to a lesser

extent upon the manifestations of the primary damage. This has been sufficiently stressed hereinbefore. It is true that in these instances it is probably more correct to speak not of a predominant influence of the different forms of protection upon the course of the recuperatory processes, but merely of a better-defined manifestation of the protective action during this period of the radiation reaction.

In addition to the noted features of similarity, there are also found certain differences in the nature of the protective action of the screening of an organ during irradiation and that of the protective action due to subsequent replacement of the organ. Such a difference was ascertained by us, in particular, in the case of a comparison of the results of spleen screening and spleen transplantation. Whereas, with absolutely lethal dosages of irradiation, the screening of the spleen produces a protective effect (see Table I), the transplantation of the spleens does not affect in these instances the survival rate. We have carried out a special limited-scale experiment (with 15 animals of the experimental series and 14 control animals) in which irradiation was effected with a minimum absolutely lethal dosage (700r) and the implantation of spleens did not produce any beneficial effect. The cause of the observed difference in the effects of screening and transplantation is in our opinion the fact that implantation of spleen begins to exercise its beneficial effect upon the irradiated animals at a much later time than is the case with screened organ. Since with absolutely lethal dosages the radiation reaction proceeds at a much more rapid rate and is more intensive than following lethal dosages, the effects of an implanted spleen, which manifest themselves unquestionably subsequent to the transplantation operation, do not have sufficient time to alter the course of this reaction.

However, the ineffectiveness of spleen transplantation upon application of absolutely lethal dosages is due not only to this reason. Another cause, in our opinion, is the fact that spleen implantation produces a beneficial effect primarily by enhancing the resistance of the irradiated organism to secondary infections (which will be considered in detail hereinafter), whereas the death of animals irradiated with absolutely lethal dosages is not due to infectious diseases.

The facts under discussion are directly connected with the question concerning the nature of the protective action of implanted spleen and injected bone marrow. Is it due to cellular structures or to some humoral factor produced by the resolving introduced tissues? Our data, which show the importance of an adaption of the spleens for the effectuation of a protective action, indicate that it is associated with cellular structures. At the same time, the fact that in the case when no adaption of the implanted organ has taken place, a certain beneficial effect is still produced indicates the possibility that a humoral factor may also be involved in the protective effect. We believe that the same is also indicated by the data relating to injection of bone marrow, wherein it can hardly be assumed that the protective action is associated with adaption of the cells of this tissue. Thus, we are inclined to assume a dual nature of the protective effects of spleen transplantation and bone marrow injection.

In conclusion, let us consider briefly the possible mechanism of the beneficial action of spleen transplantations and bone marrow injections. The nature of the effect of spleen transplantation upon the irradiated animals is determined by the functional significance of this organ. The role of the spleen as a hematopoietic organ in mice is very great; therefore

there is every reason for assuming that the adapted spleens normalize the hematopoiesis in animals having been subjected to irradiation. We assume that of no lesser importance in the protective effect are the adapted spleens as a source of enhancement of the protective functions of the organism in its control of secondary infections. The role of the spleen in this connection is well known; it is associated with the phagocytic activity of this organ and with its capability to produce antibodies.

Our data provide a certain indication that the positive effects of spleen implantation is associated with decreased probability of the occurrence of infectious diseases. It is precisely to this that there can be attributed the above-mentioned divergence of the survival curves relating to the experimental and the control groups of mice, only 8 days after the irradiation, and the fact associated therewith of a greater life duration of the mice which died in the control group rather than those which died in the experimental group.

It is known that death of the animals during the 2 to 3 weeks after irradiation and later is usually due to a development of bacteriemia. It is also possible that implantation of spleen has a stimulating effect on the leucopoietic function of the natural spleen of the recipient. The latter is indicated by the large amount of leukocytes not only in the blood of mice with adapted spleens but also in the blood of mice which have undergone implantation but in which adaption of the implanted spleen has not yet occurred. An increasing number of leukocytes within the circulating blood enhances the defensive capabilities of the organism in its control of infections.

On considering the question of the possible mechanism of the protective action of the bone marrow, it should be stated that in a number of features it is probably similar to the mechanism of the action of spleen implants. It is true that in this instance the stimulating action of the introduced bone marrow upon the hematopoiesis of the irradiated animals becomes of primary importance. Increased hematopoiesis of the irradiated animals determines the enhancement of their resistance to detrimental factors and, in particular, to secondary infections. This provides an explanation of the fact that the protective action of bone marrow, as well as that of spleen implantation, is associated with a decrease in mortality, not during the initial period of the radiation reaction but during the later course.

A conclusive elucidation of the mechanism of the protective action of spleen implants and bone-marrow administration requires further experimental investigations.

Conclusions

1. Screening of hematopoietic organs (spleen and bone marrow) sharply increases the rate of survival of mice having been subjected to general Xray irradiation.

2. The protective action of the screening of hematopoietic organs is revealed most clearly in the course of the recuperatory processes.

3. A method has been worked out for spleen transplantation (from young mice to their mothers) which ensures a high degree of adaption of the transplanted organs. Histological analysis of the adapted spleens has shown that they are normally functioning hematopoietic organs.

4. Transplantation of spleen from nonirradiated to irradiated animals renders milder the course of the radiation reaction in the irradiated animals and increases their rate of survival.

5. Intravenous administration of bone marrow of nonirradiated mice to irradiated mice also produces a beneficial effect upon the course of the radiation reaction and increases the rate of survival of animals having been exposed to Xray radiation.

6. A comparison of the protective action of the screening of hematopoietic organs, spleen implants, and administration of bone marrow has revealed the following facts:

(a) The extent of protective action is about the same in all the investigated cases.

(b) The protective action is especially clearly manifested in all the tested procedures during the recuperative processes.

7. The protective action of spleen transplants and intravenous administration of bone marrow is presumably associated with:

(a) a stimulation of the hematopoiesis in the irradiated animals.

(b) Increased defensive capabilities of the irradiated animals in the control of secondary infections.

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ADDENDUM

TABLE I

TIME OF DEATH OF MICE FOLLOWING IRRADIATION IN THE EXPERIMENTS WITH SCREENING OF THE SPLEEN. TOTAL IRRADIATION OF THE CONTROLS

Dosage (r)	Group	Total number of animals	Number of animals that died	Day of death 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
500	Experiment	27	8	1 1 1 1 1 1 1 1
	Control	24	14	1 1 3 1 1 2 2 1 1 1 1
600	Experiment	17	6	1 1 1 2 1 1 1
	Control	17	15	3 1 1 2 5 1 1 1
800	Experiment	21	14	2 3 5 1 1 1 1
	Control	20	20	4 10 3 3

TABLE II

TIME OF DEATH OF MICE FOLLOWING IRRADIATION IN THE EXPERIMENTS WITH SCREENING OF ONE EXTREMITY (EXPERIMENT I) AND TWO EXTREMITIES (EXPERIMENT II). TOTAL IRRADIATION OF THE CONTROLS

Dosage (r)	Group	Total number of animals	Number of animals that died	Day of death 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
600	Experiment I	34	10	1 1 2 2 1 1 1 1 1
	Experiment II	34	4	1 1 1 1
	Control	34	28	2 3 5 6 1 8 1 1 1
700	Experiment I	23	16	8 4 1 1 1 1 1
	Control	24	24	9 2 5 4 3 1

TABLE III

CHANGES IN WEIGHT OF SURVIVING MICE IN THE EXPERIMENTS WITH SCREENING OF THE SPLEEN. TOTAL IRRADIATION OF THE CONTROLS

Dosage (r)	Group	Days of weighing	0	+4	+8	+12	+16	+20	+24	+28
500	Experiment	Mean weight	22.6	21.0	20.8	22.8	23.1	23.0	24.2	24.1
		Weight index	100.0	93.0	92.1	100.9	102.3	101.8	107.1	106.7
		Number of animals	19	19	19	19	19	19	19	19
	Control	Mean weight	23.9	21.3	20.0	20.4	20.8	21.1	22.8	22.4
		Weight index	100.0	89.2	83.6	85.4	87.0	88.3	95.4	93.7
		Number of animals	10	10	10	10	10	10	10	10
600	Experiment	Mean weight	24.6	20.8	21.9	22.9	23.7	24.4	24.8	25.7
		Weight index	100.0	84.5	89.1	93.1	96.4	99.2	100.8	104.5
		Number of animals	11	11	11	11	11	11	11	11
	Control	Mean weight	24.8	21.2	20.0	20.8	19.7	22.2	22.7	24.0
		Weight index	100.0	85.5	80.6	83.9	79.4	89.5	91.6	96.7
		Number of animals		2	2	2	2	2	2	2
800	Experiment	Mean weight	23.7	19.6	18.9	19.5	21.1	21.8	22.2	23.0
		Weight index	100.0	82.8	79.8	82.4	89.2	92.1	93.7	97.2
		Number of animals	7	7	7	7	7	7	7	7
	Control	Mean weight	All the animals died							
		Weight index								
		Number of animals								

TABLE IV

CHANGES IN WEIGHT OF THE SURVIVING MICE FOLLOWING IRRADIATION IN THE EXPERIMENTS WITH SCREENING OF ONE (EXPERIMENT I) AND TWO EXTREMITIES (EXPERIMENT II). TOTAL IRRADIATION OF THE CONTROLS

Dosage (r)	Group	Days of weighing	0	+4	+8	+12	+16	+20	+24	+28
600	Experiment I	Mean weight	21.6	19.7	20.9	21.8	23.1	23.5	23.8	24.7
		Weight index	100.0	83.5	96.5	101.0	106.8	108.3	110.2	114.6
		Number of animals	24	24	24	24	24	24	24	24
	Experiment II	Mean weight	21.9	19.2	21.1	21.6	22.7	23.3	23.6	24.2
		Weight index	100.0	87.9	96.3	98.9	104.2	103.4	108.6	110.2
		Number of animals	30	30	30	30	30	30	30	30
	Control	Mean weight	21.8	18.6	18.6	18.4	19.4	19.8	21.3	22.6
		Weight index	100.0	86.9	86.9	84.4	88.9	91.9	97.4	103.0
		Number of animals	6	6	6	6	6	6	6	6
700	Experiment I	Mean weight	26.8	22.9	24.9	24.7	25.9	26.4	26.5	27.0
		Weight index	100.0	85.5	92.6	92.2	96.5	98.5	99.1	100.1
		Number of animals	7	7	7	7	7	7	7	7
	Control	Mean weight	All the animals died							
		Weight index								
		Number of animals								

TABLE V

TIME OF DEATH OF MICE FOLLOWING IRRADIATION. EXPERIMENT: IRRADIATION OF MICE WITH TRANSPLANTED SPLEENS.

CONTROL: IRRADIATION OF MICE NOT SUBJECTED TO SPLEEN TRANSPLANTATION

Dosage (r)	Group	Total number of animals	Number of animals that died	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
500	Experiment	71	18				2			3	3	2		3	1	2		
	Control	63	36				2	1	2	3	1	8	4	3	2	2	1	2

TABLE V (end)

Dosage	Group	Total number of animals	Number of animals that died	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
500	Experiment	71	18				1		1									
	Control	63	36	1	1								1		1			1

TABLE VI

CHANGES IN WEIGHT OF MICE FOLLOWING IRRADIATION. EXPERIMENT: MICE TO WHICH BONE MARROW WAS INTRAVENOUSLY ADMINISTERED AFTER IRRADIATION. CONTROL: MICE TO WHICH NO ADMINISTRATION OF BONE MARROW WAS MADE

Group	Number of investigated animals	Days of weighing	0	+4	+8	+12	+16	+20	+24	+28
Experiment	55	Mean weight	24.6	23.3	23.6	23.8	24.7	25.2	25.3	25.5
		Index	100.0	94.7	95.9	96.7	100.4	102.0	102.4	103.6
Control	37	Mean weight	23.7	22.4	22.6	22.1	22.5	23.5	23.6	24.2
		Index	100.0	94.5	95.3	93.2	94.9	99.1	99.5	102.1

TABLE VII

CHANGES IN WEIGHT OF MICE FOLLOWING IRRADIATION. EXPERIMENT: IRRADIATION OF MICE WITH TRANSPLANTED SPLEENS. CONTROL: IRRADIATION OF MICE WITHOUT SPLEEN IMPLANTS

Group	Category of animals	Days of weighing	1	+5	+9	+13	+17	+21	+25	+29
	Surviving	Number of animals	53	53	53	53	53	53	52	52
		Mean weight	29.2	25.3	24.6	25.2	25.8	26.2	26.5	26.4
		Index	100	86.6	84.1	86.2	88.4	89.9	90.8	90.7
Experiment	Those with adopted spleens	Number of animals	30	30	30	30	30	30	30	30
		Mean weight	28.0	24.5	24.4	25.0	25.3	25.5	26.0	26.5
		Index	100	87.6	87.3	89.5	90.6	91.2	93.0	95.0
	Those without adopted spleens	Number of animals	23	23	23	23	23	23	22	22
		Mean weight	30.6	26.4	24.9	25.4	26.4	26.9	27.3	26.3
		Index	100.0	86.2	81.2	83.0	86.2	87.8	89.1	85.9
Control	Surviving	Number of animals	27	27	27	27	27	27	27	27
		Mean weight	27.9	23.4	23.8	23.9	24.4	24.5	24.8	24.4
		Index	100	84	85.4	85.7	87.5	87.7	89.9	87.5

TABLE VIII

CHANGES IN THE AVERAGE NUMBER OF LEUKOCYTES PER 1 mm^3 OF BLOOD IN MICE FOLLOWING IRRADIATION. EXPERIMENT: MICE TO WHICH BONE MARROW WAS INTRAVENOUSLY ADMINISTERED AFTER IRRADIATION. CONTROL: MICE WHICH RECEIVED NO BONE MARROW

Group	Number of animals investigated	Days of determination						
		-1	+5	+10	+15	+20	+25	+30
Experiment	19	10400	700	4090	7830	6780	7300	8260
Control	12	9430	690	1970	6020	7900	6010	5550

TABLE IX

TIME OF DEATH OF MICE FOLLOWING IRRADIATION. EXPERIMENT: IRRADIATION MICE TO WHICH A SUSPENSION OF BONE MARROW WAS ADMINISTERED

CONTROL: IRRADIATED MICE TO WHICH A LIKE AMOUNT OF BUFFER SOLUTION WAS ADMINISTERED

Dosage (r)	Group	Total number of animals	Number of animals that died	Day of death																										
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
500	Experiment	104	49					2	5	6	9	8	1	3	1	3	1		2		1	1		2	1			1	1		1	
	Control	104	67					3	8	4	6	15	9	5	6	2	4				1		2						2			

TABLE X

CHANGES IN THE AMOUNT OF LEUKOCYTES IN THE BLOOD OF MICE FOLLOWING IRRADIATION. EXPERIMENT: IRRADIATED MICE WITH TRANSPLANTED SPLEENS. CONTROL: IRRADIATED MICE WITHOUT SPLEEN IMPLANTS

Group	Category of animals	Days of determination	-1	+5	+10	+15	+20	+25	+30
Experiment	Those with adopted spleens	Average number of leucocytes per 1 mm^3 of blood	5991	1002	2457	8261	9150	7703	8150
		Number of animals	12	14	14	14	14	14	14
Experiment	Without adopted spleens	Average number of leucocytes per 1 mm^3 of blood	4254	1181	1701	6941	8059	5931	6345
		Number of animals	9	11	11	11	11	11	11
	Surviving	Average number of leucocytes per 1 mm^3 of blood	5766	663	1246	4786	7110	6146	5965
		Number of animals	15	17	16	15	15	16	16

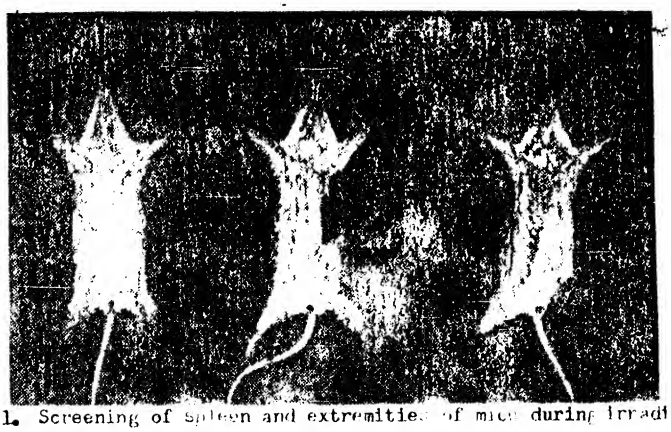

Figure 1. Screening of spleen and extremities of mice during irradiation.

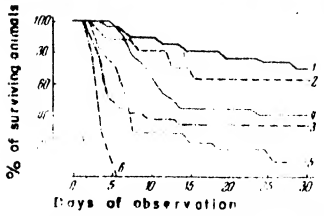

Figure 2. Survival of mice following irradiation carried out with screening of the spleen.

On screening of spleen: 1, dosage 500 r; 2, dosage 600 r; 3, dosage 800 r. Total irradiation: 4, dosage 500 r; 5, dosage 600 r; 6, dosage 800 r.

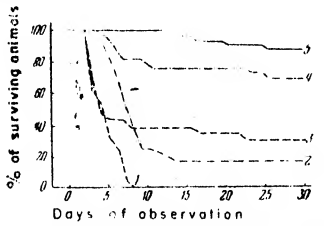

Figure 3. Survival of mice following irradiation carried out with screening of the bone marrow.

Total irradiation: 1, dosage 700 r; 2, dosage 600 r.

With screening of two extremities: 5, dosage 600 r.

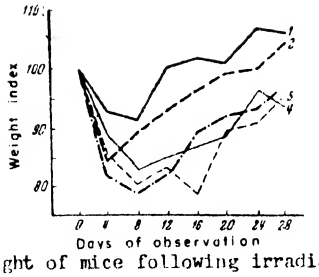

Figure 4. Changes in weight of mice following irradiation carried out with screening of the spleen.

On screening of the spleen: 1, dosage 500 r; 2, dosage 600 r; 3, dosage 800 r. Total irradiation: 4, dosage 500 r; 5, dosage 600 r.

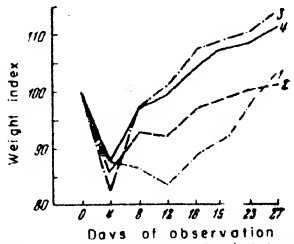

Figure 5. Changes in weight of mice following irradiation carried out with screening of the bone marrow.

Total irradiation: 1, dosage 600 r. On screening of one extremity: 2, dosage 700 r; 3, dosage 600 r. On screening of two extremities: 4, dosage 600 r.

Figure 6. Dissected mouse with adapted spleens.

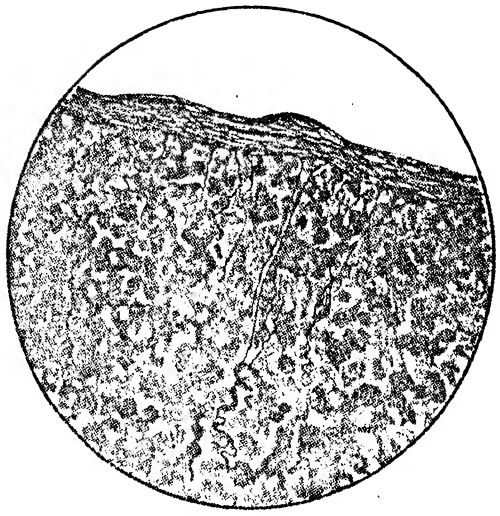

Figure 7. Connective tissue capsule of an adapted spleen with inwardly extending trabeculae.

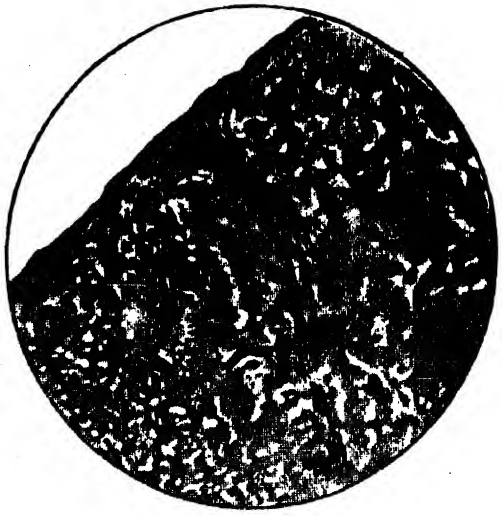

Figure 8. Connective tissue capsule of natural spleen with inwardly extending trabeculae.

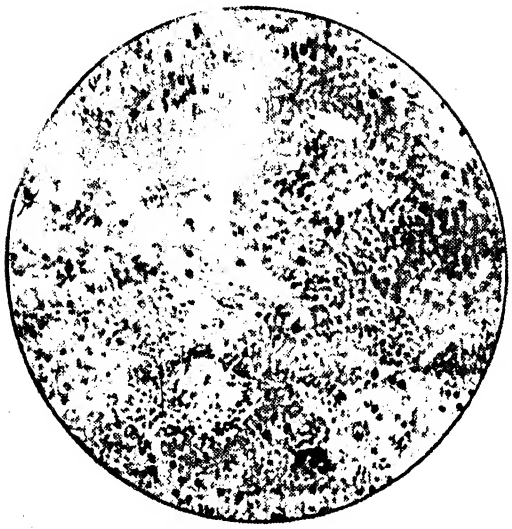

Figure 9. Malpighian bodies of an adapted spleen.

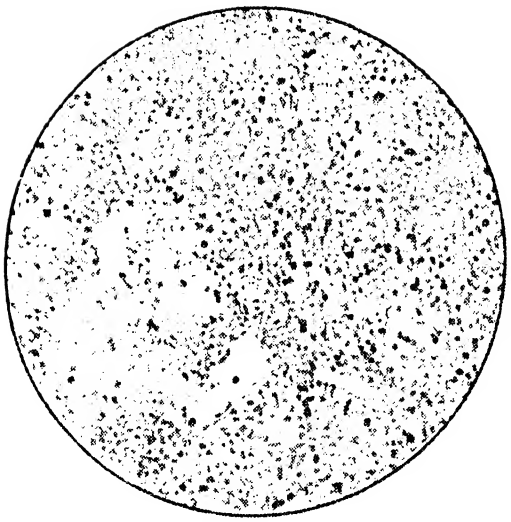

Figure 10. Malpighian bodies of a natural spleen.

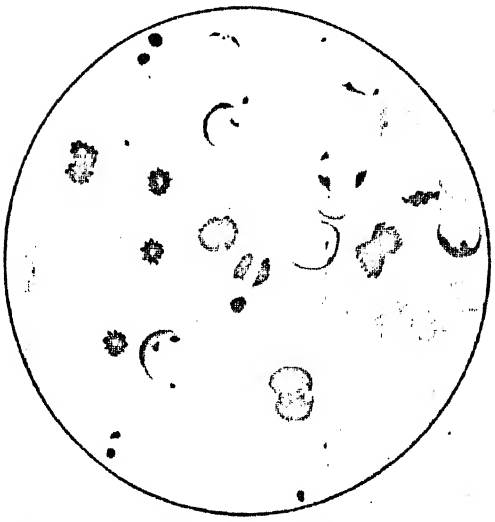

Figure 11. Mitoses in cells of an adapted spleen.

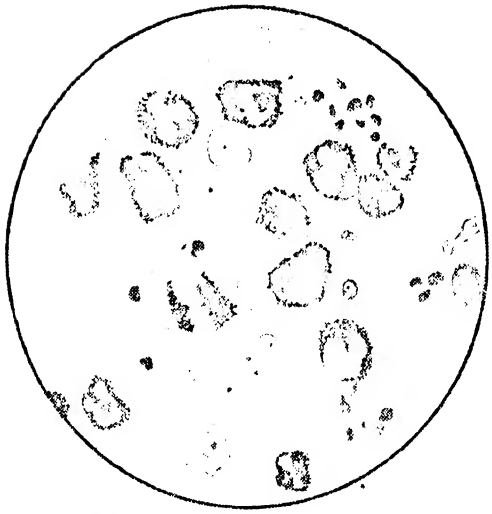

Figure 12. Various stages of development of eosinophiles in an adapted spleen.

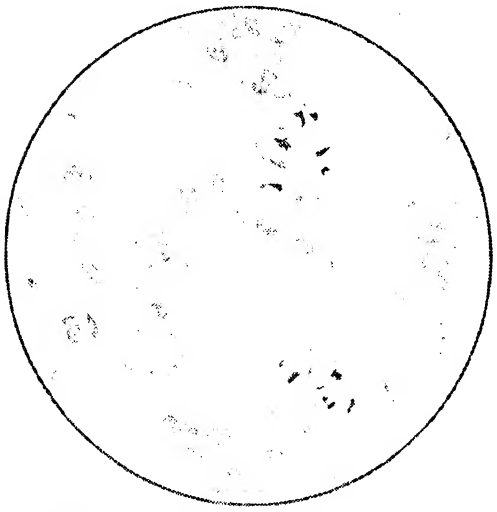

Figure 13. Megakaryocytes in an adapted spleen.

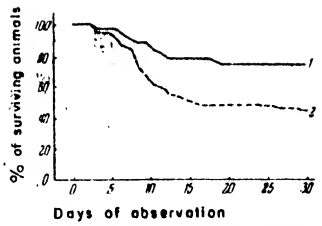

Figure 14. Survival of mice with implanted spleens following irradiation.

1, mice with implanted spleens; 2, control mice.

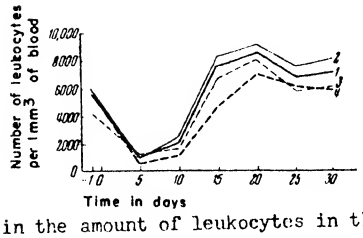

Figure 15. Changes in the amount of leukocytes in the blood of mice with implanted spleens following irradiation.

1, mice with implanted spleens; 2, mice with adapted spleens; 3, mice with unadapted spleens; 4, control mice.

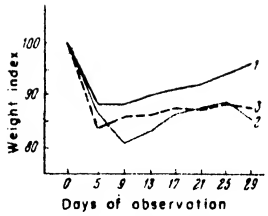

Figure 16. Change in weight of mice with implanted spleens following irradiation.

1, mice with adapted spleen; 2, mice with unadapted spleens; 3, control mice.

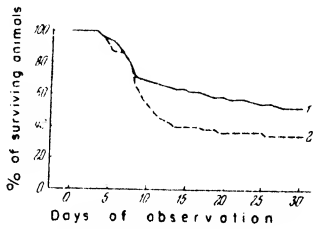

Figure 17. Survival of mice following irradiation on administration of bone marrow.

1, mice to which bone marrow had been administered;

2, control mice.

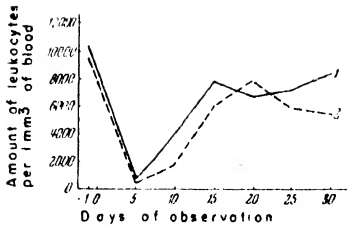

Figure 18. Changes in the amount of leukocytes in the blood of mice following irradiation upon administration of bone marrow.

1, mice to which bone marrow had been administrated;

2, control mice.

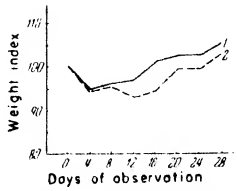

Figure 19. Changes in weight of mice following irradiation upon administration of bone marrow.

1, mice to which bone marrow had been administered;

2, control mice.

STERILIZING ACTION OF IONIZING RADIATION ON MAMMALS

N. I. Nuzhdin,
N. I. Shapiro,
O. N. Petrova

COMMUNICATION I

EFFECT OF XRAY IRRADIATION ON THE FERTILITY OF MALE MICE

INTRODUCTION

The present paper is the first of a series of our investigations concerned with the study of the regularities of the action of penetrating radiations on the fertility of mammals. The problems with which these investigations are concerned are by no means of a novel nature in radioactive substances and Xrays researchers have devoted considerable attention to the effects of these factors on fertility. Hence it is quite natural that a large amount of literature data relating to this question has become available.

Without reviewing this literature, we nevertheless wi h to point out that the overwhelming majority of investigations which have been carried out in this field were conducted not on mammals but on other species of animals -- and even on plants. Thus, the most detailed analyses of the sterilizing action of ionizing radiation have been conducted on insects. In that instance it was shown that the sterilizing effect of ionizing radiations is connected with damage to the chromosomes of genital cells (Astaurov and Frolova, 1935; Ostryakova-Varshaver, 1937). A number of researchers revealed the hereditary nature of the sterility induced by the action of radiation (Neuhans, 1937; Berg, 1938).

Considerably fewer contributions are concerned with studies of the

action of radiation on the fertility of mammals. Already in the investigations appertaining to the early period of the development of radiobiology, it was shown that the fertility of mammals -- females as well as males -- constitutes an exceptionally radiosensitive test. The latter was ascertained not only by direct studies of the fertility of irradiated animals (Okinchits, 1906), but also by means of histological studies of gonads (Bergonie and Tribondean, 1901; Halberstaedter, 1905; Zaretskiy, 1908), as well as by investigations of the oestrous cycle (Schugt, 1928; Geller, 1930).

Unfortunately this early work involves substantial defects: first, they lack dosimetric data, and, secondly, the biological effect of irradiation as a rule was not expressed quantitatively. All this led to the determination of only a general picture of the sterilizing effect of irradiation in mammals without revealing any quantitative regularities and mechanisms which result in lowering the fertility in animals having been subjected to radiation exposure.

In more recent contributions these defects have been eliminated to a considerable extent, but therein likewise no determination has been made of the quantitative regularities of the sterilizing action of radiation on mammals. It should also be taken into consideration that in the overwhelming majority of recently conducted researches, studies were carried out on the sterilizing action of the local irradiation of gonads or even of the irradiation of sperms in vitro (Rokitskiy, Neuganz, and Kardymovich, 1934; Rokitskiy, Papalashvili, Khritova, and Shekhtman, 1934; Rokitskiy, Papalashvili, and Shekhtman, 1935). Yet one of the most important tasks is not the study of the effects of penetrating radiations upon local exposure, but rather on the action upon the entire organism.

To the foregoing it should be added that in those isolated contributions in which the objects of studies were mammals that had been subjected to total irradiation no sufficiently thorough study was made of the fertility of experimental animals. Thus, for example, studies were made of the sterilizing action of Xrays on animals, but no account was taken in the course thereof of the nature of development and the fertility of their offspring. Moreover, no determination was made of the regularities of fertility recuperation. The sterilizing effects of Xrays were studied, but no investigation was made of the histological changes in the genital glands, etc. Finally, in no single instance has the attempt been made to compare data which characterize the radiation reaction in mammals with the sterilizing effect of irradiation.

All these circumstances have made it thoroughly necessary to study further the sterilizing action of total Xray irradiation in mammals. The present paper is concerned with the study of the regularities of the sterilizing action of total Xray irradiation on male mice.

Material and Procedure

As the object of investigation were utilized Sexually mature, 2 to 4-months-old male mice of strain A which had been subjected to a single total Xray irradiation. The conditions of irradiation were as follows: voltage, 160 kv; current intensity, 5 ma; filters, 0.75 mm Al +0.5 mm cu; focal distance, 40 cm; dosage intensity, 15.3 r/min; exposure dosage, 200 and 400r. The animals were irradiated in small batches. In each series the mice were subjected to an irradiation of 200r, as well as 400r; each series was provided with controls of the same age which consisted of animals not previously exposed to radiation. Irradiation of the mice was conducted in wooden boxes divided by wood partitions into 12 compartments, each measuring 3 x 7 cm. (Details concerning the irradiation procedure are found in the paper by N. I. Shapiro and N. I. Nuzhdin

"Effects of Different Dosages of Xray irradiation on the survival of mice," which is included in the present symposium). The control animals were also placed in boxes for a length of time equal to that employed for the irradiation. After irradiation, the males were placed individually in glass jars having a capacity of 10 lit.

The sterilizing action of Xrays on the males was evaluated from the results of their mating with nonirradiated females. The females were brought together with the males at different time intervals following the irradiation. In addition to direct studies of fertility, histological analyses were made of the genital glands of the irradiated animals. Also studied was the embryonic and postembryonic development of the offspring sired by males which had undergone radiation exposure.

During the entire investigation, the experimental and control animals were kept under identical conditions. The feed included oats, milk, bread, wheat grits, hemp or sunflower seeds. Vegetables and greens given to the animals included carrots, dandelion leaves, plantain leaves, oat sprouts. In winter and spring the mice were given fish-liver oil and yeast.

Course of the Radiation Reaction in Mice Subjected to Xray Exposure

It is known that ionizing radiation induces in animals radiation injury which develops earlier and in a more acute form with increasing dosage of exposure (Shapiro and Nuzhdin, 1955).

In male mice subjected to an irradiation of 400r the outward symptoms of radiation damage began to appear 4 to 5 days following exposure. The mice became less lively, their fur was ruffled, and a sharp decrease

in appetite was observed. Somewhat later, disturbances of the gastrointestinal tract set in. Over the first 12 days following irradiation, a considerable loss in weight of the animals occurred. In a number of cases conjunctivitis developed. Upon application of the 200r dosage the symptoms of radiation injury became manifest somewhat later and were of a less acute nature. Not only the onset of the disease, but also the duration of its acute stage, depended upon the exposure dosage. While with a dosage of 200r the outward symptoms of radiation injury subsided completely in an overwhelming majority of the animals by the end of the first month, upon an irradiation of 400r, to which part of the animals were subjected, the general condition had not yet fully returned to normal by the end of the month. The animals were less lively and their fur remained ruffled.

It has been stated before that one of the symptoms of radiation injury is the loss in weight of the animals. This symptom characterizes to a certain extent the general condition of the organism during the course of radiation injury. We have conducted individual determinations of the weight of the experimental animals and of the controls every fourth day during the first month following irradiation. The absolute values of the changes in weight of the males which survived following irradiation are listed in Table 1.

Figure 1 shows the curves which represent the dynamics of the weight changes of males subjected to an irradiated of 200r and 400r.

In these graphs the weight of the animals is expressed as weight indices (the initial weight being equal to 100). The curves show clearly that in mice subjected to 200r the radiation injury is not accompanied by a loss in weight. They show only a certain lag in weight increase as compared with the controls. Upon a dosage of 400r,

there becomes apparent already on the fourth day a sharp decrease in weight. By the twelfth day the mean weight of the animals reaches its minimum, after which it begins to increase gradually, reaching by the twenty-first day the initial level. Although thereafter the weight of the animals continues to increase, nevertheless, one month after the irradiation the animals of this group lag considerably in weight behind the controls.

In those animals in which the radiation syndrome was most sharply manifested, the disease terminated in death. The data of Table 2 show that of the 71 animals subjected to an irradiation of 200r, seven died which constitutes 9.9±3.54%; while of the 24 animals irradiated with 400r, 48 died, i.e., 51.1±5.16%. No instances of death occurred in the group of control animals. These figures indicate that the number of animals that die increases with increasing irradiation dosage.

The correlation between lethal effect and the dosage of Xray irradiation is clearly revealed by the curves which represent the time of death of the experimental animals (Figure 2). Table I of the addendum shows the factual data on the basis of which these curves have been plotted. Examination of the curves reveals that with a dosage of 200r, as well as with a dosage of 400r, the first instances of death among the mice occur on the fifth to sixth day following exposure, and that the most frequent instances of death were observed within the interval between the fifth and tenth day following irradiation. In the case of mice which died after being exposed to 200r, the mean life duration was 10.9 days; in the case of those subjected to 400r it was 11.5 days. The somewhat longer duration of life of mice irradiated with 400r is due to the fact that in this group of animals, evidently because of the bacteriemia which developed in these animals after irradiation, mortalities also continued to occur after a longer interval of time.

TABLE 1

CHANGES IN WEIGHT OF THE MALES WHICH SURVIVED AFTER IRRADIATION

Number of animals and their weight	Dosage of exposure (r)	Days of weighing 0	4	8	12	16	21	25	29
Mean weight of animals (g)	200	22.5	22.9	22.8	23.2	24.3	24.7	25.3	25.7
Weight index		100.0	101.7	101.3	103.2	108.1	109.7	112.4	114.2
Number of animals weighed		40	40	40	40	39	40	40	39
Mean weight of animals (g)	400	23.4	22.7	22.2	21.8	22.6	23.3	24.1	24.5
Weight index		100.0	96.8	94.8	93.1	96.4	100.4	103.2	104.8
Number of animals weighed		30	30	30	30	30	30	30	30
Mean weight of animals (g)	con-	22.8	23.8	23.7	24.3	25.3	25.8	26.3	26.7
Weight index	trol	100.0	104.4	103.9	106.6	111.0	113.1	115.3	116.7
Number of animals weighed		46	46	46	46	46	46	46	46

TABLE 2

SURVIVAL OF IRRADIATED MALES

Exposure Dosage (r)	Total number of animals	Survived		Died		Mean life duration of animals that died (days)
		Number	Percent	Number	Percent	
200	71	64	90.1±3.54	7	9.9±3.54	10.9
400	94	46	48.9±5.16	48	51.1±5.16	11.5
Control	76	76	100.0	-	-	-

We have presented herein a description of the course of radiation injury in the experimental mice, in order to give an idea of the extent of radiation damage inflicted to the organism upon use of the above-stated dosages of Xray irradiation. These data can be utilized for making a comparison of the nature of the radiation damage to the organism, due to a direct exposure, with those consequences which become manifest in the offspring begotten by the irradiated animals.

Breeding Capability and Number of Offspring of Irradiated Males

Testing of the fertility of the males was effected at the following time intervals: (1) immediately following irradiation; (2) after one month; (3) after 3 months following irradiation. Usually each male was mated with two nonirradiated females of the same strain. Since radiation injury developed in the males which had been subjected to irradiation there was every reason for believing that soon after the irradiation these males would not breed. For this reason, in the first tests, females in the proestrous or oestrous state were kept with the males for the relatively short period of one to 2 days. In the second and third tests the females were left with the males for one week, and therefore the oestrous stage was not taken into account. After being separated from the males, the females were kept in groups of six in glass jars of 10-lit capacity. Pregnant females were housed individually. All instances of pregnancies and births were recorded.

The experiments have shown that a single total Xray irradiation of males in dosages of 200 and 400r results in a decrease of their fertility. This decrease in fertility is associated above all with a reduced breeding of the irradiated males (Table 3). The begetting of but a single litter by the male being tested was utilized as a criterion of procreativeness.

The data listed in Table 3 show that immediately after irradiation the breeding capability of the males which had been subjected to exposure is reduced only slightly. The number of males which begot offspring amounts to 42.2±5.42% at 400r, 37.5±6.99% at 200r, and 57.1±6.62% among the controls. One month following exposure, the breeding capability of the males decreases sharply, especially following a dosage of 400r. In this case, the number of males which begot offspring decreases to 16.7±7.61%, as compared with 84.8±6.25% among the controls (M_{dif} = 67.3±9.8%). Although the acute stage of radiation injury has subsided by then (body weight and blood picture of the mice approximate normal), the general condition of the animals is such that breeding does not occur readily. Three months after the exposure the situation alters. The breeding capability of the males in both variants of the experiment virtually does not differ from that of the controls, as is shown by the following figures: the number of males that produced offspring is 68.2±9.93% with 200r, 71.4±9.86% with 400r, and 75.0±8.12% in the control group. The small differences between the experimental and control series are statistically unreliable.

The reduced breeding capability of males which had been subjected to irradiation is also evidenced by the date which characterize the efficacy of mating of nonirradiated females with the experimental males.

TABLE 3

BREEDING CAPABILITY OF THE IRRADIATED MALES

Exposure Dosage (r)	Time lapse following irradiation: Immediately			One month later			Three months later		
	Males tested	Progeny: Number	Progeny: Percent	Males tested	Progeny: Number	Progeny: Percent	Males tested	Progeny: Number	Progeny: Percent
200	48	18	37.5±6.99	26	20	76.9±8.27	22	15	68.2±9.93
400	83	35	42.2±5.42	24	4	16.7±7.61	21	15	71.4±9.86
Control	56	32	57.1±6.62	33	28	84.8±6.25	18	21	75.0±8.12

TABLE 4

NUMBER OF EFFECTIVE MATINGS OF FEMALES WITH IRRADIATED MALES

Exposure Dosage (r)	Time lapse following irradiation: Immediately				After one month				After 3 months			
	Males tested	Number of matings	Effective: Number	Effective: Percent	Males tested	Number of matings	Effective: Number	Effective: Percent	Males tested	Number of matings	Effective: Number	Effective: percent
200	48	93	24	25.8±4.54	26	51	31	60.8±6.84	22	42	22	52.4±7.71
400	83	159	40	25.2±3.49	24	48	4	8.3±3.98	21	41	19	46.3±7.79
Control	56	108	38	35.2±4.60	33	64	44	68.7±5.80	28	56	29	51.8±6.68

The data of Table 4 show that immediately after irradiation the percent of effective matings of the animals which had been exposed to radiation is lower than that among the controls.

One month after the exposure, the effectiveness of the mating of females with males which had been irradiated with a dosage of 400r differs sharply from the effectivenss of the mating among the controls. In this case, the number of effective matings in the experiment amounts to 8.3±3.98%, as compared with 68.7±5.80 among the controls. Reliability of the difference: M_{dif}= 60.4±7.03. With a dosage of 200r, the percent of effective matings in tests made at this time is close to that of the controls. Finally, after 3 months following irradiation, the effectiveness of mating in the case of males of the group subjected to a dosage of 400r, although approximating the normal rate, still remains somewhat lower.

Of great importance in the characteristic of the decrease in fertility of irradiated males is the reduction in the number of their offspring. The data on the number of offspring in litters produced at different time intervals following exposure to radiation are shown in Table 5. From these data it is apparent that the average number of offspring (summative, including the live and stillborn) in litters sired by irradiated males is considerably smaller than in the controls. This decrease is especially sharply manifested following dosages of 400r (in litters produced immediately following irradiation, as well as one month after irradiation). After a lapse of 3 months the average number of offspring in litters of both experimental variants approximates that of the controls; nevertheless, in the case of males subjected to a dosage of 400r it remains somewhat lower. Of unquestionable interest are the data showing the average number of viable offspring per litter. The corresponding data which are also included in Table 5 show that in this case the differences between the average number of offspring per litter becomes still more pronounced between the control and the experimental animals.

TABLE 5

AVERAGE NUMBER OF OFFSPRING IN LITTERS SIRED BY IRRADIATED MALES

Exposure dosage (r)	Time lapse following irradiation: Immediately			One month later			3 months later		
	Number of litters	Number of offspring	Average number of offspring per litter (M±m)	Number of litters	Number of offspring	Average number of offspring per litter (M±m)	Number of litters	Number of offspring	Average number of offspring per litter (M±m)
200	33	200	6.1±0.30	46	279	6.1±0.28	50	344	6.9±0.30
400	51	226	4.4±0.23	8	28	3.5±1.12	44	277	6.3±0.32
Control	48	343	7.1±0.31	46	295	6.4±0.34	50	359	7.2±0.33
				Excluding stillborn					
200	33	191	5.8±0.31	46	271	5.9±0.31	50	340	6.8±0.31
400	51	214	4.2±0.25	8	25	3.1±0.92	44	266	6.0±0.35
Control	48	342	7.1±0.31	46	290	6.3±0.34	50	356	7.1±0.34

The data cited indicate the sterilizing effect of Xray irradiation on male mice, which is the more pronounced the greater the exposure dosage. At the same time these data indicate that the lowering of the fertility of irradiated males is of a temporary nature, since on lapse of a definite length of time following irradiation the fertility of the males is restored. The time of recuperation and its extent depend upon the irradiation dosage.

Worthy of special consideration in regard to the offspring of irradiated males is the considerable number of stillbirths. Table 6 shows data on the number of stillbirths in regard to litters sired by irradiated males. It can be seen from this table that the litters sired by irradiated males immediately after irradiation, as well as one month thereafter, following exposure to either dosage of irradiation, relate to a larger proportion of stillbirths in comparison with the controls, and that this increased proportion is directly dependent upon the exposure dosage. With a dosage of 400r, the frequency of stillbirth is greater than with a dosage of 200r. In litters sired 3 months after irradiation the number of stillbirths was found to be equal to that encountered among the controls only in the group subjected to a dosage of 200r. The offspring of males subjected to an irradiation of 400r relate to a higher percentage of stillbirths than that found in the controls even 3 months after the exposure.

As an explanation for the occurrence of stillborn fetuses among the offspring of irradiated males, the assumption has been advanced that, due to the decreased number of individuals per litter, larger embryos are developed. The larger size of the embryos leads in turn to difficulties during birth which result in the death of mice during delivery (Snell, 1933).

TABLE 6

NUMBER OF STILLBORN MICE AMONG THE OFFSPRING OF IRRADIATED MALES

Exposure Dosage (r)	Time Lapse Following Irradiation: Immediately			After 1 month			After 3 months		
	Number of offspring	Stillborn		Number of offspring	Stillborn		Number of offspring	Stillborn	
		Number	Percent		Number	Percent		Number	Percent
200	200	9	4.5±1.47	279	8	2.9±1.00	344	4	1.2±0.59
400	226	12	5.3±1.49	28	3	10.7±5.84	277	11	4.0±1.17
Control	343	1	0.3±0.29	295	5	1.7±0.75	359	4	1.1±0.55

To determine the correctness of this assumption, we have calculated the average number of offspring per litter containing stillborn mice, and per litter comprising only living mice. The results of these calculations, shown in Table II of the addendum, reveal that occurrence of stillbirths does not depend on the size of the litter. Thus, the only possible cause of the occurrence of numerous stillbirths among the offspring of irradiated males is the damage to the genital cells of the latter.

Analysis of the Causes of a Decreased Number of Offspring of Irradiated Males

As was pointed out hereinbefore, one of the principal indices of the reduced fertility of irradiated males is the decreased number of their offspring. To determine the causes of the reduced number of offspring it is necessary to consider first of all their viability. The presence of stillborn fetuses among the offspring of irradiated males provided a reason for believing that the decreased number of offspring may be due to the death of embryos during the early stages of development. This possibility is also indicated by the literature data (Snell, Bodemann, and Hollander, 1934; Russel, 1952). With the view of determining the correctness of this assumption, dissections were carried out on pregnant females which had been impregnated by the males immediately after the irradiation of the latter.

In order to be in a position to investigate embryos developing at more or less equal stages, it was of importance to know the time of fecundation. For this purpose females in proestrous or oestrous state were kept with the males for one day. The occurrence of a mating was determined by the presence, in the vagina of the female mouse, of a so-called stopper. In the absence of a stopper, vaginal smears were

taken daily to follow the oestrous cycle of the females. The presence of a dioestrus over a period of 7 to 8 days gave reason for assuming that the female was pregnant. On the eighth to ninth day following removal from the males, the pregnant females were put to sleep by means of ether narcosis. Both horns of the uterus were opened and the number of embryos determined. A record was made of the total number of viable embryos and also of the number of nonviable. The latter were differentiated by their relatively small size and also by a peculiar greyish-brown coloration.

The results of these dissections are shown in Table 7.

As is apparent from the table, about 6% of the dead embryos were observed in the control. In the experiment, wherein the males were subjected to 200r, the percentage of dead embryos reaches 36%. Upon a dosage of 400r, it is 47%. Consequently, in the last-mentioned instance almost one-half the fertilized ova perished after development had started.

Data concerning the average size of the litter during different stages of development are shown in Table 8. Calculations of the mean values was based on the number of 8- to 9-day embryos and the number of offspring at birth. In birth instances the average number given is that as determined for all offspring, as well as for only the viable.

TABLE 7

VISIBILITY OF EMBRYOS ON THE EIGHTH TO NINTH DAY OF DEVELOPMENT

Exposure dosage (r)	Pregnant females investigated	Number of embryos found	Embryos			
			Viable		Nonviable	
			Number	Percent	Number	Percent
200	21	145	93	64.1±3.98	52	35.9±3.98
400	29	188	100	53.3±3.64	88	46.7±3.64
Control	21	161	151	93.8±1.90	10	6.2±1.90

TABLE 8

MEAN SIZE OF LITTER DURING DIFFERENT STAGES OF DEVELOPMENT

Exposure dosage (r)	Average Number of Young Per Litter			
	8- to 9-day-old embryos		At birth	
	Total (M±m)	Viable (M±m)	Total(M±m)	Viable (M±m)
200	8.2±0.42	6.6±0.63	5.7±0.32	5.4±0.33
400	7.4±0.44	5.5±0.47	4.4±0.29	4.1±0.30
Control	8.3±0.61	7.9±0.58	7.3±0.33	7.3±0.33

The data cited show that the death of embryos at different stages of development results in a decrease of the number of viable offspring sired by the irradiated males. The same data indicate a direct relationship between the death rate of the developing offspring and the dosage of exposure.

From a comparison of the average size of the litter, calculated on the basis of the viable 8- to 9-day old embryos with the average value based on viable offspring at birth, it follows that death during the early stages of embryogenesis is encountered more frequently than during the later stages. Thus, in the presence of a dosage of 400r prior to the eighth or ninth day of pregnancy, about 3 times as many embryos die as during the period between the eighth to ninth day and the termination of pregnancy.

Thus, the conclusion can be reached that the principal cause of the decrease in the number of offspring sired by irradiated males is the death of the embryos at different stages of embryogenesis.

The problem of the sterilizing action of Xrays is to a considerable extent a problem of the development of offspring derived from animals which have been subjected to irradiation. Hereinafter we shall see that this statement applies only to offspring derived from genital cells which at the time of irradiation were already-formed spermatozoids or were at stages approximating the latter.

Changes in Weight of the Testes and Accessory Genital Glands as an Index of the Sterilizing Action of Xrays

The weight of the testes, seminal vesicles, and prostate constitutes to a certain extent a reflection of their functional state. To carry out the weighing, the animals of the experimental as well as

of the control series were dissected at different intervals of time following irradiation. The first dissection was carried out one day after irradiation; the second after 15 days; the third after one month; and, finally, the fourth and last, 3 months after irradiation. Right and left testes were weighed separately, upon which their total weight was computed. The seminal vesicles were weighed with the prostate.

During the course of the investigation attention was attracted by the decrease in volume of the testes in experimental animals at the middle, and especially at the end, of the acute period of the course of radiation injury. Following a lapse of 3 months after radiation exposure, the weight of the testes of irradiated animals became relatively restored. The data characterizing the changes in weight of male genital glands following irradiation are shown in Table 9, which includes, in addition to the absolute values, the ratio of the average weight of the organ to the average body weight, expressed in percent.

The data listed in Table 9 show that one day after irradiation the weight of the testes in the experimental group of mice differs but slightly from those of the controls. By the middle of the course of radiation injury the testicular weight of the irradiated animals is found to have sharply decreased, and after 30 days, i.e., by the end of the acute period of radiation injury, the decrease in weight becomes still more substantial. We note that exposure to 400r is more effectual than exposure to 200r. After 3 months following exposure, the irradiated animals show increasing average testicular weight, but not equaling that of the control animals.

TABLE 9

CHANGES IN THE WEIGHT OF TESTES AND ACCESSORY GENITAL GLANDS OF IRRADIATED MICE

Exposure dosage	Males investigated	Mean weight of mice (g)	Mean weight of testes (mg)	Index	Mean weight of accessory genital glands (mg)	Index
			24 hours after irradiation			
200	8	21.7	140.4	0.65	119.9	0.55
400	8	22.1	152.0	0.69	138.1	0.62
Control	8	21.9	166.8	0.76	125.8	0.57
			15 days after irradiation			
200	6	23.7	118.6	0.50	154.7	0.65
400	7	19.1	100.6	0.53	98.8	0.52
Control	6	24.7	184.0	0.74	194.7	0.79
			One month after irradiation			
200	6	26.1	96.0	0.37	196.2	0.75
400	8	24.3	70.8	0.29	175.0	0.72
Control	6	26.2	183.2	0.70	231.5	0.88
			3 months after irradiation			
200	5	25.5	185.6	0.73	260.8	1.02
400	5	26.6	167.0	0.63	256.6	0.96
Control	5	29.1	260.8	0.90	350.0	1.20

The dynamics of changes in weight of the seminal vesicles and the prostate differs from that of the testes. The initial weight values for the seminal vesicles and the prostate were sufficiently similar in the three groups being compared. Fifteen days after irradiation, the weight of the seminal vesicles and the prostate had decreased only in animals subjected to an irradiation of 400r. Animals subjected to an irradiation of 200r not only show no decrease in the weight of these organs, but show an increase in their bodily weight which is less, however, than that observed in the controls. One month after irradiation animals of all the groups show further increase in the weight of the seminal vesicles and the prostate. The same is also true as concerns the last period of observation, i.e., 3 months after the exposure. During the last two periods of observation the weights of the seminal vesicles and the prostate in the different groups of animals are found to be distributed in the following order. The greatest weight values are found in the control animals; those subjected to 200r follow thereafter; and, finally, the animals subjected to 400r. Thus, the dynamics of the weight of the genital glands and their accessory organs is in good agreement with the data relating to fecundity which have been reported hereinbefore, viz., the Xrays damage the genital glands of males, and this damage is more pronounced with greater dosage of exposure. At the same time, the data obtained indicate a recuperation with a certain length of time from the damage inflicted.

Figure 3 shows a graph which represents the dynamics of changes in the body weight, testicular weight, and the weight of the accessory genital glands of the irradiated animals. The indices utilized in this graph (in contrast to those of Table 9) are the ratios of mean body weight and the weight of the organs of the animals which had been

subjected to irradiation, to the corresponding mean weight values in the control animals which are taken as being equal to 100. From a comparison of these curves, it follows that the male genital glands are especially sensitive to irradiation. Thus, while the body weight of animals subjected to an irradiation of 400r shows a maximum decrease of 23%; that of the accessory genital glands decreases 50%, and the weight of the testes decreases 60%.

We note in addition that the drop in the weight of the testes still continues at the time when the body weight of the animals begins to increase (and when, according to our other data, recuperation is also shown by the blood picture). This evidences a greater radiosensitivity of the testes and also the fact that recuperative processes occur therein more slowly than those upon which the general condition of the animal depends.

Histological Analysis of the Testicular Structure of Irradiated Animals

Known at the present are a large number of researches concerned with sufficiently-detailed studies of the damages to the testes of mammals (especially of rodents) induced by ionizing radiations (Zedgenidze, Kotik, Larionov, et al., 1936; Bloom, 1948; Eschenbrenner and Miller, 1950; Fogg and Cowing, 1952; Shaver, 1953). The histological analysis of the testes of irradiated animals which we have carried out is limited to the general picture of the radiation damage of this organ. Such an analysis was indispensible for a comparison (at the selected time intervals) of the damage pictures revealed in the microscopical structure of genital glands, with those damages disclosed by means of a hybridous analysis of fertility.

In connection with the task involved fixation of the testes of animals was carried out at the following points of time: (1) after 12 hours, (2) after 24 hours, (3) after 15 days, (4) after one month, and (5) after 3 months, following irradiation. The first, second, fourth, and fifth time intervals of fixation coincide with the time intervals of the fertility tests of the irradiated males, which makes it possible to form an opinion concerning the course of spermatogenesis at the time of the tests. At each of the time intervals two animals were killed in both the experimental and control series. The testes were subjected to a fixation procedure in Zenker's solution, as modified by Maksimov. The sections were 4 to 6 μ thick. Staining was done with iron hematoxylin, according to the Haydenhein method.

(a) Histological Structure of Testes 12 Hours After Irradiation

Twelve hours after the exposure, the general microscopic picture of the structure of the testes of animals subjected to an irradiation of 200r, as well as to one of 400r, does not differ essentially from that of the control animals. The tubuli are well filled-out, and their diameter does not differ from that of the seminal tubuli of the control animals. The tubuli contain seminal cells at different stages of spermatogenesis. Some spermatocytes in the testes of irradiated animals are in the process of mitosis.

On a more detailed study of the sections of the testes of irradiated mice it is possible, even during this period, to detect the action of radiation. Thus, among the cells adjoining the basement membrane, there are cells having pycnotic nuclei which possibly constitute spermatogonia which were in the state of division at the time of exposure. Pycnotic alterations also affect the nuclei of spermatocytes. In these

instances they acquire a uniform, fairly intensive stain coloration. Sometimes the chromatin is conglomerated into a cluster and is distributed in the shape of a crescent along the periphery of the nucleus. Found fairly frequently at the locations of spermatogonia and spermacytes are rounded particles of unstained plasma which apparently constitute the remnants of degenerated cells. Also found in the testes of irradiated animals are gigantic multinuclear spermatides. Some of them bear clear traces of degeneration. The described alterations are encountered somewhat more frequently in the testes of animals subjected to an irradiation of 400r than in those subjected to 200r.

(b) Histological Structure of Testes 24 Hours After Irradiation

At this time, the testes of animals irradiated with a dose of 400r show an appreciable decrease in the amount of spermatogonia. Otherwise, the histological picture of the testes of animals previously subjected to exposure does not differ from that described above.

(c) Histological Structure of Testes After 15 days Following Irradiation

After 15 days subsequent to exposure there is noted in the testes of irradiated animals a decrease in the number of germ cells. At the same time the degree of devastation of seminal tubuli indicates a direct correlation with the dosage of exposure. With a dosage of 200r, degeneration is not as extensive as in the case of a 400r dosage. The testes of animals irradiated with a dose of 200r are characterized by the presence of a small number of cells constituting the initial stages of spermatogenesis, viz., spermatogonia and spermacytes. The principal mass of germinal tissues is composed of spermatides and spermatozoids, which is adequately illustrated by the microphotograph shown in Figure 4.

With a dosage of 400r, the devastation of seminal tubuli has proceeded considerably further. Conspicuous is the lack of uniformity in the degree of damage to different seminal tubuli (Figure 5). Tubuli are found, the histological structure of which is similar to that observed in the case of a dosage of 200r. In these the principal mass of germinal cells consists of spermatides and spermatozoids. Present on the other tubuli are essentially Sertoli cells and only a small amount of spermatides and spermatozoids. Spermatogonia and spermacytes are very rarely encountered. Figure 6 shows a microphotograph illustrating the structure of a testes of an animal of the same age which had undergone no Xray irradiation.

(d) Histological Structure of Testes One Month After Irradiation

An interesting picture is that of testes subjected to fixation one month after irradiation. The diameter of seminal tubuli in irradiated animals is less than in the controls. At the same time there is manifested therein a relationship with the dosage, since the diameter of the tubuli is less in testes of animals subjected to a larger dosage of Xrays (400r), as compared with the other experimental group subjected to a lesser dosage (200r). The different composition of the germinal cells in the testes of animals irradiated with different dosages also emphasizes the considerable difference in the effectiveness of these dosages. With a dosage of 200r almost all the seminal tubuli are uniform in cellular composition (Figure 7). They contain cells in all stages of spermatogenesis, but the amount of spermatozoids is very small. The picture observed indicates that in the testes of animals subjected to 200r an intensive process of spermatogenesis restoration takes place one month after exposure.

In the testes of animals subjected to 400r a sharp lack of uniformity is observed in the structure of seminal tubuli (Figure 8). Some tubuli

are filled only with Sertoli cells, the nuclei of which are clearly visible at the basis of the membrane. Vacuolation of the plasma of these cells is clearly manifested. The structure of other seminal tubuli recalls the tubuli of testes of animals irradiated with 200r. In these, restoration of spermatogenesis is in progress. Not infrequently these tubuli have a reserve of spermatogonia. Spermacytes are not disposed in two or three rows as in the controls, but are scattered at random. A number of tubuli show a structure that is of an intermediate nature: a varying number of spermacytes are scattered among the Sertoli's cells. Testes of animals irradiated with 400r are characterized during this period by an almost complete absence of spermatides and spermatozoids.

(e) Histological Structure of Testes After 3 months Following Irradiation

The histological structure of testes of animals irradiated with 200r (Figure 10) approximates that of the testes of the control animals. The seminal tubuli bear almost no traces of damage due to irradiation. Only occasionally are there encountered gigantic multinuclear spermatides. In animals irradiated with 400r there also takes place by this time a restoration of spermatogenosis (Figure 11). However, the histological picture of the structure of testes in these animals differs somewhat from that of the controls (Figure 12). Thus, the lumina of seminal tubuli in the experimental animals are larger, which is apparently connected with the lesser number of cells in the late stages of spermatogenesis, viz., spermatides and spermatozoids.

Thus, the above-presented data show that by action of the irradiation a sharp disruption of spermatogenesis occurs in the male mice. The disruption is associated on the one hand with damage to the spermatogenetic cells at different stages of spermatogenesis, and on the other with its

temporary cessation. The latter is apparently caused by the fact that the spermatogonia retained by the irradiated animals temporarily lose the capability of undergoing division; since in all the cases which we have investigated spermatogonia were present in the testes, although sometimes in a very small amount, the restoration of spermatogenesis is apparently the result of a restoration of the capacity of spermatogonia to undergo division.

The lesser degree of damage following irradiation with 200r results in a situation wherein recuperative processes begin at a time when the available reserves of germinal cells have not yet been fully depleted, and therefore a period of total sterility, if it occurs in these animals, is of only brief duration. With a dosage of 400r this period is more prolonged, which is evidenced by the incomplete restoration of spermatogenesis even after 3 months following exposure.

Offspring Derived from Irradiated Males After the Restoration of their Fertility

Since the histological analysis has shown that Xray irradiation results in damage to germinal cells present at different stages of spermatogenesis, the question arose concerning the radiosensitivity of these cells from the standpoint of the effects upon the offspring. In this connection, it was of particular interest to trace the effect produced by the radiation on genital cells which at the time of irradiation are in the stage of spermatozoids or at stages close thereto, and also whether or not irradiation affects genital cells formed during the process of recuperation.

Known are a large number of researches conducted mostly on insects, in particular on Drosophila, in which it was shown that Xray irradiation

damages primarily the nuclei of spermatozoids or of cells which are at stages close to these. Nuclei of cells present at earlier stages of spermatogenesis (spermatogonia) suffer to a considerably lesser degree from the radiation damage (Shapiro, 1931; Serebrovskaya and Shapiro, 1935). Similar data were subsequently secured in regard to mammals (Hertwig, 1935). Thus, every reason existed for assuming that the viability of offspring derived from irradiated males after restoration of their fecundity will be substantially greater than that of the offspring derived from the same males directly after their irradiation.

The data which we have analyzed hereinbefore provide in part an answer to the question concerning the effects of an irradiation of spermatozoids on the viability of the offspring. It is known that the supply of sperma contained in the testis appendage of the male mouse is sufficient for only four to five copulations (Hertwig, 1938). Hence, it was of interest to determine the size of the first litters sired by irradiated males and the number of stillborn. In testing the fertility of males, females were brought together with them successively at different time intervals following the irradiation, and in every instance records were kept of the effectiveness of the mating. Thus, we were in a position to segregate the first litters irrespectively of the fact whether they were sired immediately after irradiation or at a later time. Data showing the average size of only the first litters and the number of stillbirths occurring therein are shown in Tables III and IV of the addendum. A comparison of these data with those shown in Tables 4 and 5 indicates that the number of offspring in the first litters is less, and the number of stillbirths is greater by comparison with the subsequent litters. The data of the above-mentioned tables also permit reaching the following conclusion: the average size of the first litter and the number of stillbirths do not depend upon whether this litter was sired immediately after

irradiation or at a later date. These data indicate the high sensitivity of spermatozoids, and the stages close thereto, toward irradiation (as concerns the effects upon the offspring), which is in good agreement with the large number of facts concerning the action of ionizing radiation on alteration of hereditary characteristics of animals and plants (Shapiro, 1931; Serebrovskaya and Shapiro, 1935; Hertwig, 1935).

To provide an answer to the question concerning the effects of irradiation on the genital cells formed during the process of reparation, a special investigation was carried out. Each of the irradiated males (dosage 400r) sired up to seven to eight litters. Records were kept of the size of the litters and the number of stillbirths. It was assumed that if the first litters are produced by genital cells which at the time of irradiation are present in the form of spermatozoids, the subsequent litters will be produced by germinal cells which at the time of irradiation were at earlier stages of the spermatogenesis. If the damaging action of Xrays affected only the mature genital cells or the cells approximating them in their stage of development, then, in the presence of an increasing consecutive number of litter, its size will increase and the stillbirths will become less numerous. If, on the other hand, the damaging action of Xrays affects to the same extent germinal cells which were at earlier stages of gametogenesis all the litters sired by irradiated males will be about the same size and will include the same proportion of stillbirths. The results of these experiments are shown in Table 10 and Figure 13.

TABLE 10

AVERAGE NUMBER OF OFFSPRING AND STILLBIRTHS IN A CONSECUTIVE SERIES OF LITTERS

Consecutive numbers of litters	Number of litters	Total number of offspring	Average number of offspring per litter (M±m)	Stillborn offspring	
				Number	Percent
1	59	277	4.7±0.30	18	6.5±1.48
2	23	122	5.3±0.38	4	3.4±1.64
3	15	96	6.4±0.61	1	1.0±1.02
4	11	71	6.5±0.81	2	2.8±1.96
5	5	37	7.4±1.03	-	-
6	7	46	6.6±0.43	1	2.2±2.16
7	5	37	7.4±1.40	-	-
8	1	8	8.0	-	-
			Control		
1-8	182	1240	6.8±0.17	13	1.0±0.28

Table 10 includes data showing the average number of offspring and the number of stillbirths in the consecutive litters. Figures characterizing the average size of the litter show that, from the first to the fourth litter, the average number of offspring per litter (being relatively small) increases and remains thereafter at about the same level as is observed with the controls.

The graph of Figure 13 shows the distribution of litters sired by the irradiated and the control males, depending upon their size (numerical data are shown in Table V of the addendum).

The graph shows clearly that the size of the first two litters sired by the irradiated males is smaller than those sired by the control animals (the differences are statistically reliable: $M_{dif\ 1} = 2.1 \pm 0.34$ and $M_{dif\ 2} = 1.5 \pm 0.41$), whereas the size of the subsequent litters approximated that of the controls. It should be noted that the first two litters sired by irradiated males are not only less numerous in comparison with the subsequent litters but the stillbirths therein are encountered oftenest.

This permits arriving at the following two conclusions. (1) The most sensitive stage of spermatogenesis as concerns the effects upon the offspring is the stage of spermatozoids. It is precisely from cells, which at the time of the irradiation were at the stage of spermatozoids or close thereto, that there could be produced the first two litters in which the number of offspring is sharply reduced and the greatest number of stillbirths occur. (2) Mature genital cells formed during the reparation process do not carry gross traces of the damaging action of irradiation. This is evidenced by the normal size of the subsequent litters and a number of stillbirths approximating that sired by the controls.

These conclusions are important not only from a theoretical standpoint;

possibly they are of no lesser practical significance. Although we are not justified to apply directly to humans the data secured by means of investigations of laboratory animals, we should still take them into account. If subsequently, these data will be confirmed in other mammals, the following conclusion of practical importance can be arrived at: in cases of radiation therapy applied to the region of testes there is less to be feared from the damaging action of the irradiation upon the issue of the patient if this issue is derived from genital cells which were produced after the recovery of fertility.

Postembryonic Development of Offspring Sired by Irradiated Males

As was previously stated, decreased number of offspring derived from males subjected to irradiation is due to the death of the embryos at different stages of their development. In this connection, the question arose as to the normalcy of the course of postembryonic development of mice sired by these males, and whether the traces of the damaging action of penetrating radiation affect the postembryonic development of the offspring. To provide an answer to this question a follow-up was carried out on the postembryonic development of offspring resulting from the mating of males subjected to an irradiation of 200 and 400r with nonirradiated females. Investigations were conducted not only on the offspring of litters sired soon after the irradiation (first and second litters), in which by analogy with the embryonic development a maximum effect of the irradiation could be assumed, but also on litters sired 3 months after the irradiation (sixth and seventh litters), in which the number of offspring and the percentage of stillbirths did not differ from those of the controls, and hence a damaging effect of irradiation could hardly be assumed to be present. Observation of the animals were carried out through the first month and a half of their postembryonic development, i.e., from the time of birth to sexual maturity. As a result, it was

ascertained that the survival of the members of the first generation sired by the irradiated males does not differ from that of the controls (offspring of first and second litters, as well as those of sixth and seventh litters).

In processing the materials, separate account was kept of the mortality of the offspring of different sex. No differences in the survival rate of males and females were detected. A determination was also made of the ratio of sexes among the offspring of irradiated males. The literature contains conflicting data on this subject. According to some investigators (Parkes, 1925; Kalmus, Metrakos, and Silverberg, 1952) the ratio of sexes among the offspring of irradiated males deviates from the normal. According to the data of other researchers, no deviations from the normal are found in the numerical ratios of the sexes (Trasher and Metrakos, 1953). In our experiments, determinations were made of the number of females and males in litters sired at different intervals of time following the irradiation of the males. Table 11 shows the data relating to the ratio of sexes in litters sired immediately after irradiation of the males. These data show that the offspring of irradiated and of control males contained equal numbers of either sex. Similar results were obtained upon analysis of the offspring sired one month and 3 months following irradiation.

Notwithstanding the large number of litters analyzed, we have found no morphological deviations in the development of the offspring of the animals of the experimental groups, as compared with that of the controls.

We have also studied the growth of animals sired by the irradiated males. It is known that growth regularities are usually studied by means of the weight change curves of the developing animals.

TABLE 11

SEX RATIOS OF THE OFFSPRING OF IRRADIATED MALES (LITTERS SIRED IMMEDIATELY AFTER IRRADIATION)

Irradiation dosage (r)	Total number of offspring	Including		% of males
		males	females	
200	148	79	69	53.4±4.10
400	110	60	50	54.5±4.75
Control	150	74	76	49.3±4.10

The mice were weighed at the following time intervals: first, fifth, thirteenth, twenty-first, twenty-eighth, thirty-fifth, and forty-second day after birth. These time intervals were selected for the following reasons. The fifth day coincides with the appearance of fur, the thirteenth with the opening of the eyes, twenty-first with beginning of independent food intake, and the twenty-eighth with separation from the dam. Since the growth characteristics of mice depend upon the sex and number of animals in the litter, a corresponding grouping of the data was carried out in the processing data. Thus, the average weight of the animals during the different postembryonic periods of development was calculated separately for the females and for the males. Separate processing was carried out on the data relating to litters containing from one to three, from four to six, and from seven to nine offspring. Data were derived which characterize the growth of offspring sired immediately after irradiation of the males, i.e., of the first two litters and also of that sired 3 months after the exposure, i.e., the sixth and seventh litters. Numerical indices relating to the changes in weight of the animals are shown in their entirety in Tables VI, VII, VIII, and IX of the addendum.

Here we confine ourselves to a consideration of data limited to offspring sired immediately after irradiation. Figure 14 shows the curves

of growth of the animals, which reveal upon examination a great similarity between experimental and control groups in all three series being compared. The data cited leave no doubt that the postembryonic development of mice sired by the irradiated males does not differ from that of mice derived from nonirradiated parents.

On comparing the data on postembryonic development of mice sired by the irradiated males with analogous data relating to their embryonic development, we note the following interesting regularity. While the embryonic development of offspring sired by irradiated males bears the mark of the damaging action of the radiation, the postembryonic development is free from these effects. Evidently the gross damage arising in the genital cells due to the action of irradiation manifests itself during the process of embryogenesis, and the individuals which pass through this peculiar filter are found to be practically normal.

Conclusions

1. Xray irradiation of males has the following results.

(a) Decreased mating capability of the males

(b) Decreased number of offspring in litters resulting from mating with nonirradiated females.

2. The offspring of irradiated males includes a large number of stillbirths.

3. Decrease in the number of offspring of irradiated males occurs due to the death of the embryos during different stages of their development. Thus, the problem of the sterilizing action of Xrays is partially a problem of the development of the offspring of irradiated males.

4. Changes in weight of the testes can serve as a qualitative index of the sterilizing action of Xrays.

5. The greater the exposure dosage of Xrays, the greater their effect on the fertility of the irradiated males.

6. After the lapse of one to 3 months following irradiation, the fertility of the males becomes restored. This recuperation proceeds at a rate which increases with decreasing dosage of exposure.

7. Restoration of testes proceeds slower than the overall recuperation of the irradiated organism.

8. Mice resulting from the fertilization of ova by spermatozoas developing from regenerated germinal cells do not differ in viability from the controls. In other words, the regenerated genital cells bear no gross traces of the damaging action of radiation.

9. Postembryonic development of the offspring sired by the irradiated males reveals no deviation from the normal.

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ADDENDUM

TABLE 1

DISTRIBUTION OF ANIMALS THAT DIED BY DAYS OF OBSERVATION

Exposure dosage (r)	Number of animals that died	Days of observation																											
		0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	24-25	25-26	26-27	27-28	28-29	29-30
200	7	-	-	-	-	-	2	-	-	1	1	1	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-
400	48	-	-	-	-	-	3	2	6	7	5	9	1	4	-	2	1	-	1	-	1	1	4	-	-	-	1	-	-	-	-
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Mean duration of life (days)
200	10.9
400	11.5
Control	-

TABLE II

AVERAGE NUMBER OF OFFSPRING IN LITTERS WHICH INCLUDED OR DID NOT INCLUDE STILLBIRTHS

Exposure dosage (r)	Litters Including Stillbirths			Litters Not Including Stillbirths		
	Number of litters	Number of offspring	Average number of offspring per litter	Number of litters	Number of offspring	Average number of offspring per litter
200	5	32	6.4	50	296	5.9
400	10	58	5.8	49	219	4.5
Control	2	16	8.0	60	424	7.1

TABLE III

AVERAGE NUMBER OF OFFSPRING IN LITTERS SIRED BY IRRADIATED MALES. (BASED ON THE FIRST LITTERS)

Exposure dosage (r)	Time Following Irradiation: Immediately			After 1 month			After 3 months			Total		
	Number of litters	Number of offspring	Average number of offspring per litter (M±m)	Number of litters	Number of offspring	Average number of offspring per litter (M±m)	Number of litters	Number of offspring	Average number of offspring per litter (M±m)	Number of litters	Number of offspring	Average number of offspring per litter (M±m)
200	25	143	5.7±0.32	21	133	6.3±0.45	9	52	5.8±0.78	55	328	6.0±0.26
400	39	172	4.4±0.29	5	16	3.2±1.56	16	89	5.9±0.62	59	277	4.7±0.30
Control	36	263	7.3±0.33	19	119	6.3±0.60	7	58	8.3±1.06	62	440	7.1±0.30
				Excluding Stillbirths								
200	25	136	5.4±0.33	21	132	6.3±0.46	9	52	5.8±0.78	55	320	5.8±0.27
400	39	161	4.1±0.30	5	14	2.8±1.21	15	84	5.6±0.63	59	259	4.4±0.29
Control	36	262	7.3±0.33	19	116	6.1±0.60	7	58	8.3±1.06	62	436	7.0±0.30

TABLE IV

NUMBER OF STILLBORN MICE IN LITTERS OF IRRADIATED MALES (BASED ON THE FIRST LITTERS)

Exposure dosage (r)	Time Following Irradiation: Immediately			After 1 month			After 3 months			Total		
	Offspring investigated	Stillborn Number	Stillborn Percent	Offspring investigated	Stillborn Number	Stillborn Percent	Offspring investigated	Stillborn Number	Stillborn Percent	Offspring investigated	Stillborn Number	Stillborn Percent
200	143	7	4.9±1.81	133	1	0.7±0.72	52	-	-	328	8	2.4±0.85
400	172	11	6.4±1.87	16	2	12.5±0.27	89	5	5.6±2.44	277	18	6.5±1.48
Control	263	1	0.4±0.40	119	3	2.5±1.43	58	-	-	440	4	0.9±0.45

TABLE V

DISTRIBUTION OF LITTERS, SIRED BY IRRADIATED MALES, ACCORDING TO THEIR SIZE

Exposure dosage (r)	Litters		Number of offspring per litter: 1	2	3	4	5	6	7	8	9	10	11	12	13	Total number of litters
200	1 and 2	Number	7	5	8	16	18	9	9	5	3	2	-	-	-	82
		%	8.5	6.1	9.7	19.5	22.0	11.0	11.0	6.1	3.7	2.4	-	-	-	100.0
400	From 3	Number	-	4	1	2	5	4	10	11	3	2	2	-	-	44
	to 8	%	-	9.1	2.3	4.5	11.4	9.1	22.8	25.0	6.8	4.5	4.5	-	-	100.0
Control	All	Number	2	10	6	10	15	30	33	38	20	11	5	1	1	182
		%	1.1	5.5	3.3	5.5	8.3	16.5	18.1	20.8	11.0	6.0	2.7	0.6	0.6	100.0

TABLE VI

CHANGES IN WEIGHT OF OFFSPRING SIRED IMMEDIATELY AFTER IRRADIATION OF THE MALE

Exposure dosage (r)	Number of litters	Average number of offspring per litter	Number	Days of weighing: 1	5	13	21	28	35	42
Litters numbering 1 to 3 offspring,♀♀										
200	1	2.0	2	1.5	3.8	6.9	10.5	13.9	16.4	17.5
400	10	2.7	14	1.5	3.4	7.5	10.5	15.1	18.7	20.5
Control	7	2.6	10	1.6	3.6	6.3	10.1	13.5	16.7	18.6
Litters numbering 4 to 6 offspring,♀♀										
200	14	4.9	43	1.5	3.3	6.6	9.7	13.5	17.0	18.1
400	10	5.3	23	1.6	3.3	6.6	9.3	12.5	16.2	18.8
Control	12	5.6	37	1.4	2.9	6.1	8.5	12.1	15.3	16.8
Litters numbering 7 to 9 offspring,♀♀										
200	7	7.3	25	1.3	3.1	5.3	7.7	10.9	14.3	15.8
400	3	7.3	10	1.6	3.4	5.8	8.5	10.1	15.8	16.7
Control	13	7.7	45	1.5	2.8	5.4	8.2	11.7	14.7	15.9

TABLE VII

CHANGES IN WEIGHT OF OFFSPRING SIRED IMMEDIATELY AFTER IRRADIATION OF THE MALE

Litters numbering 1 to 3 offspring, ♂♂

Exposure dosage (r)	Number of litters	Average number of offspring per litter	Number,	Days of weighing: 1	5	13	21	28	35	42
200	3	2.7	8	1.8	2.8	5.9	8.6	12.6	16.6	18.1
400	12	2.6	17	1.5	3.3	7.2	10.6	15.2	19.1	22.1
Control	5	2.8	9	1.7	3.4	7.9	11.0	14.4	17.7	20.9

Litters numbering 4 to 6 offspring, ♂♂

200	13	4.9	32	1.5	3.3	6.6	9.8	13.2	17.6	19.3
400	11	5.2	24	1.6	3.3	7.2	9.7	13.8	18.2	20.7
Control	12	5.6	30	1.4	2.9	6.1	9.1	13.3	16.9	18.8

Litters numbering 7 to 9 offspring, ♂♂

200	8	7.5	34	1.3	3.1	5.3	7.8	11.9	15.3	17.2
400	3	7.3	12	1.6	3.4	6.0	9.1	11.5	17.3	19.5
Control	13	7.7	52	1.4	2.8	5.4	8.4	12.3	15.8	17.2

TABLE VIII

CHANGES IN WEIGHT OF OFFSPRING SIRED 3 MONTHS AFTER IRRADIATION

Exposure dosage (r)	Number of litters	Average number of offspring per litter	Number	Days of weighing						
				1	5	13	21	28	35	42
Litters numbering 1 to 3 offspring, ♀♀										
200	2	2.0	3	1.9	3.5	10.3	11.4	14.5	16.9	23.0
400	1	3.0	1	1.8	3.3	8.4	13.6	-	19.5	21.7
Control	1	3.0	1	1.2	3.2	6.6	-	10.0	11.9	14.7
Litters numbering 4 to 6 offspring, ♀♀										
200	8	5.5	21	1.6	2.9	6.0	8.1	11.6	14.8	17.3
400	5	4.8	15	1.7	3.6	6.1	9.2	12.2	16.1	17.1
Control	7	5.4	19	1.5	2.7	5.6	8.3	11.1	14.6	17.6
Litters numbering 7 to 9 offspring, ♀♀										
200	8	7.5	27	1.4	2.6	5.5	7.3	9.7	11.4	13.6
400	6	8.0	24	1.3	2.7	5.7	7.6	10.6	12.9	14.8
Control	6	7.8	22	1.3	2.5	4.7	6.5	9.3	12.0	13.7

TABLE IX

CHANGES IN WEIGHT OF OFFSPRING SIRED 3 MONTHS AFTER IRRADIATION

Litters numbering 1 to 3 offspring, ♂♂

Exposure dosage (r)	Number of litters	Average number of offspring per litter	Number	Days of weighing 1	5	13	21	28	35	42
200	2	3.0	4	1.3	2.3	2.4	8.4	12.4	18.9	21.0
400	1	3.0	2	1.8	3.3	7.9	12.3	-	19.7	21.6
Control	2	2.0	4	1.7	4.3	8.7	10.9	14.8	19.7	23.4
Litters numbering 4 to 6 offspring, ♂♂										
200	9	5.4	28	1.5	3.1	6.0	8.5	12.2	15.3	17.6
400	5	4.8	9	1.7	3.6	6.3	9.6	12.6	16.0	18.9
Control	7	5.4	17	1.5	2.7	5.6	8.1	12.2	14.9	18.3
Litters numbering 7 to 9 offspring, ♂♂										
200	8	7.5	27	1.4	2.6	5.3	7.5	10.3	12.4	14.8
400	6	8.0	24	1.3	2.7	5.8	7.7	10.5	12.8	15.1
Control	6	7.8	22	1.3	2.5	4.7	6.6	11.3	12.5	14.4

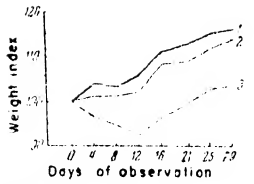

Figure 1. Changes in weight of surviving males following irradiation.

1, control; 2, dosage 200 r; 3, dosage 400 r.

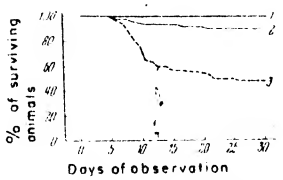

Figure 2. Survival of mice following irradiation.

1, control; 2, dosage 200 r; 3, dosage 400 r.

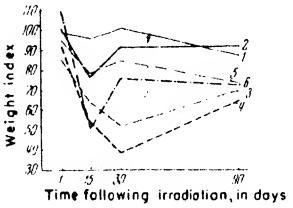

Figure 3. Changes in weight of body, testes, and accessory genital glands of irradiated males.

Body weight: 1, dosage 200 r; 2, dosage 400 r. Weight of testes: 3, dosage 200 r; 4, dosage 400 r.

Weight of accessory genital glands: 5, dosage 200 r; 6, dosage 400 r.

Figure 4. Microphotograph of testis of an animal irradiated with 200 r. Fixation 15 days after irradiated. 140 X.

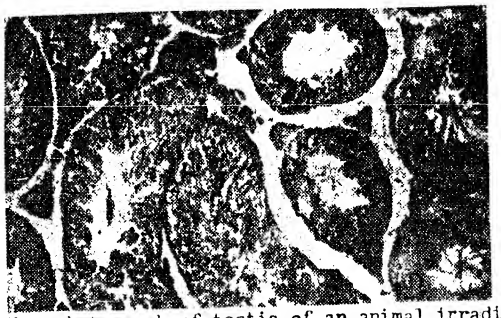

Figure 5. Microphotograph of testis of an animal irradiated with 400 r. Fixation 15 days after exposure. 140 X.

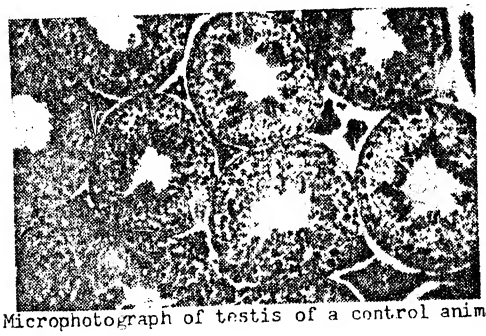

Figure 6. Microphotograph of testis of a control animal of the same age. 140 X.

Figure 7. Microphotograph of testis of an animal irradiated with 200 r. Fixation 30 days after irradiation. 140 X.

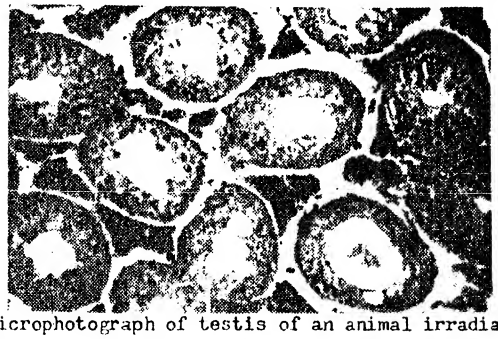

Figure 8. Microphotograph of testis of an animal irradiated with 400 r. Fixation 30 days after irradiation. 140 X.

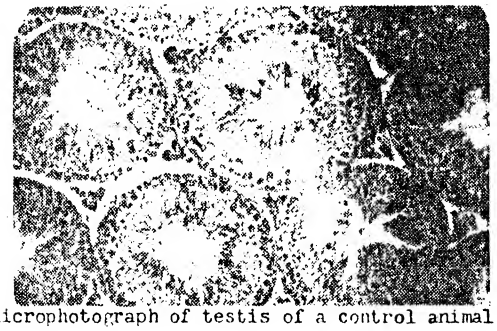

Figure 9. Microphotograph of testis of a control animal of the same age. 140 X.

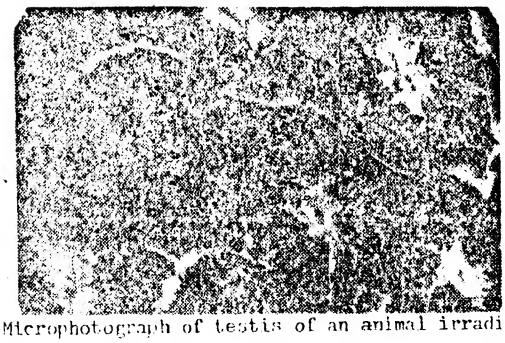

Figure 10. Microphotograph of testis of an animal irradiated with 200 r. Fixation 3 months after irradiation. 140 X.

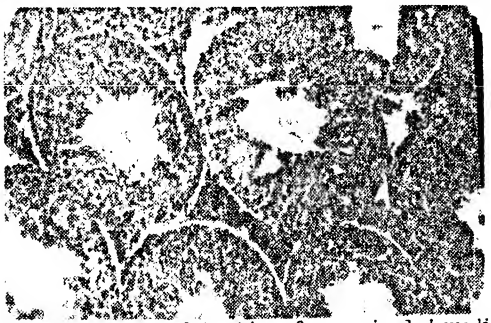

Figure 11. Microphotograph of testis of an animal irradiated with 400 r. Fixation 3 months after irradiation. 140 X.

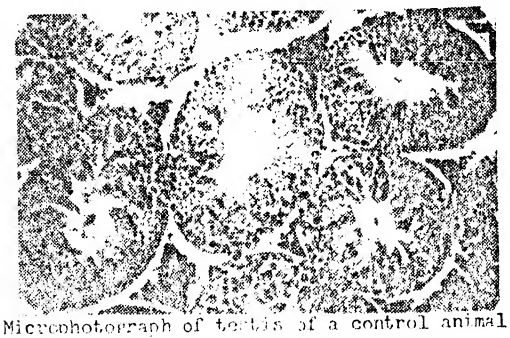

Figure 12. Microphotograph of testis of a control animal of the same age. 140 X.

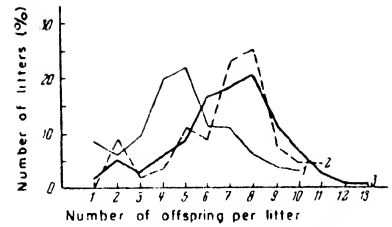

Figure 13. Distribution of litters by size.

1, first and second litters sired by males irradiated with a dosage of 400 r; 2, subsequent litters sired by males irradiated with a dosage of 400 r; 3, litters sired by males not previously subjected to irradiation.

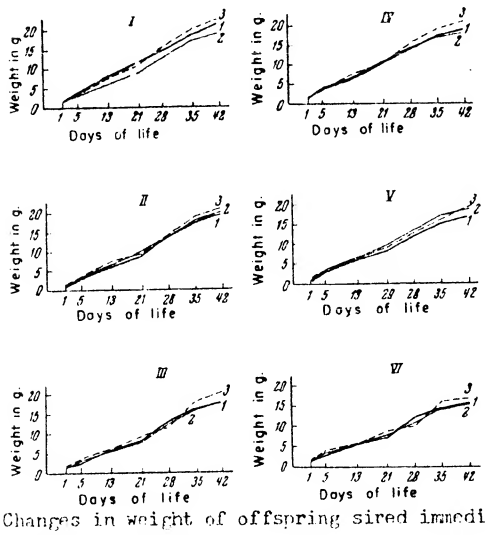

Figure 14. Changes in weight of offspring sired immediately after irradiation of the male.

1, control; 2, dosage 200 r; 3, dosage 400 r. Males: I - litters of one to 3 offspring; II - litters of 4 to 6 offspring; III - litters of 7 to 9 offspring. Females: IV - litters of one to 3 offspring; V - litters of 4 to 6 offspring; VI - litters of 7 to 9 offspring.

STERILIZING ACTION OF IONIZING RADIATION ON MAMMALS

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COMMUNICATION II

EFFECTS OF XRAY AND GAMMA IRRADIATIONS ON THE OESTROUS CYCLE OF FEMALE MICE

INTRODUCTION

Soon after the discovery of the sterilizing action of Xrays on the males of mammals (Albers-Schoenberg, 1903; Bergonie and Tribondeau, 1904), similar results were obtained in females (Bergonie, Tribondeau, and Recamier, 1905; Halberstaedter, 1905). Subsequent anatomico-histological investigations carried out on mice, rabbits, and guinea pigs (Okinchits, 1906; Specht, 1906; Bergonie and Tribondeau, 1907; Zaretskiy, 1908) have shown that in the ovaries of animals previously subjected to local Xray irradiation, there takes place, after a certain length of time following the exposure, a degeneration of follicles. Upon the use of large dosages of irradiation, the degeneration processes occur so intensively that they result in a complete elimination of all the functional structures of the ovary.

A direct test of the fertility of the irradiated animals, which was carried out for the first time by Okinchits (1906), made it possible for this author to reach the conclusion that Xray irradiation of the ovaries induces a steadily persisting barrenness of the animals, and that the exposure to small dosages of radiation leads to disruptions of the normal activities of the gonads.

Determination of the above-stated facts was of great importance in the practice of roentgenology and constituted the starting point of

experimental investigations on elucidation of the pathways and regularities of the influence of penetrating radiations on the fecundity of female mammals. Since the fecundity of animals is inseverably associated with a normal functioning of the genital glands, it is quite natural that the overwhelming majority of researches concerned with studies of the sterilizing action of radiation on females involves local irradiation of the ovaries. For the evaluation of the sterilizing action of radiation, in these investigations use was made of the histological method which permits uncovering the structural changes occurring in the tissues of the irradiated ovary (Murray, 1931; Zedgenidze, Kotik, Larionov et al., 1936; Mogil'nitskiy and Karlin, 1940; Hallberstaedter and Ickowicz, 1947; Van Eck-Vermande and Freud, 1949), as well as of the hybridous method which makes it possible to form an opinion concerning the functional activity of the ovary (Krupskiy and Ayzenberg, 1930; Snell and Ames, 1939). In addition to the two above-stated methods for studying the fertility, use was also made of a third method, viz., the study of the oestrous cycle.

The method of investigation of the oestrous cycle was worked-out on rodents (Stockard and Papanicolaon, 1917; Allen, 1922) and is based upon the fact that the genital system of the female, due to the neuro-humoral regulation, reacts synchronously to the functioning of the ovary. Processes associated with rhythmic maturation of follicles, ovulation, and formation of corpora lutea in the ovary are reflected by the state of the oviducts, and the mucosae of the uterus and vagina. Hence, from the composition of cells of a vaginal smear, it is possible to form an opinion concerning the functional state of the ovary.

In undertaking the task of studying the action of ionizing radiation on the fertility of females, we selected as the principal index of sterilizing action the disruption of the oestrous cycle. This selection

was prompted by a number of reasons of which we will mention the following.

1. The oestrous cycle is one of the convenient objective indices of the functioning of the ovaries, and thus of the fecundity of females.

2. A normal course of the oestrous cycle, as well as its disruption, can be expressed quantitatively.

3. Observations of the oestrous cycle are considerably less laborious than a direct study of the fertility of irradiated females.

4. It is possible to make a continuous study of the oestrous cycle during the entire period that follows the irradiation, whereas a continuous study of fertility, as such, is practically impossible (especially during the acute period of radiation injury).

The foregoing by no means implies that observations of the oestrous cycle fully obviate the necessity of a direct study of the fertility of females. It suffices to point out the obvious fact that the occurrence of a normal oestrous cycle in females does not constitute an absolute guaranty of their normal fertility.

Known at the present time are a relatively small number of investigations concerned with the study of the effects of ionizing radiation on the oestrous cycle. Moreover, many of them are not free from a number of procedural flaws, as a result of which the data presented therein are often a conflicting nature. Besides the fact that in almost all the investigations use was made not of a total, but of a local, exposure of the ovaries, a number of the earlier contributions lack the necessary dosimetric data as concerns the radiations (Parkes, 1926, 1927). Moreover, assessments of the disruptions of the genital cycle were based in these investigations not on observations of the cycle in its entirety, but only

on the occurrence of the oestrus stage (Schubert, 1926; Schugt, 1928). Yet it is known that the oestrus may occur in the absence of ovulation (Wolley, Fekete, and Little, 1939; Giest, Gaines, and Escher, 1941).

It must also be pointed out that in none of the contributions pertaining to this field was an attempt made to express quantitatively the disruptions of the course of the oestrous cycle, and that the authors had merely confined themselves to a description of the general picture of the impairment.

In some of the early papers even the very fact of the effects of Xray irradiation upon the course of the oestrous cycle is questionable. Thus, in the investigations wherein the method of vaginal smears was first utilized for the study of the action of Xray irradiation on the course of the oestrous cycle in mice, an ineffectiveness of radiation was reported. The irradiated animals showed a normal frequency of oestrus occurrence, and the mean duration of the cycle did not differ from that observed in the control mice (Parkes, 1927).

Conclusively demonstrated in subsequent work was the effect of irradiation on the estrous cycle -- irradiation causing substantial disruptions therein. These disruptions manifested themselves in the form of abnormal cycles (lack of the necessary sequence in the course of the stages), nontypical oestrus (in which in lieu of a purely squamous stage, the smear shows admixtures of leukocytes and epithelial cells) prolonged oestrous stage, and certain other deviations. Such disruptions in the course of the oestrous cycle upon irradiation of the ovaries were ascertained in mice, rats, and guinea pigs (Schugt, 1928; Geller, 1930; Drips and Ford, 1932; Genther, 1934).

A number of investigations discuss questions concerning the time of the occurrence of disruptions of the oestrous cycle in irradiated animals. Thus, Geller (1930) has shown that after local action upon the gonads of mice of Xrays at dosages of 200 to 500r disruptions of the oestrous cycle manifest themselves not immediately but after the lapse of period of 2 months following irradiation. After the above-stated period the vaginal smears reveal the occurrence of a prolonged squamous stage (rarely the squamae are absent or are admixed with other cells and mucus). Kullender (1953) has ascertained that in mice, 75 days after total Xray irradiation (170r), the frequency of the occurrence of the oestrus stage is reduced. Two months after Xray irradiation (54-140r), according to the data of Schugt (1928), a sharp disruption of cycle occurrence was observed in mice. This author notes that in a portion of the irradiated animals the flow reoccurred after 6 months, but was atypical. In the opinion of Zondek (1938), complete cessation of cycle occurrence in mice following local Xray irradiation is observed after 3 to 4 months.

Contrary to Geller and Kullender, who determined the occurrence of a specific latent period preceeding the manifestations of the damaging action of irradiation on the course of the oestrous cycle, Drips and Ford (1932) observed, upon local Xray irradiation of the ovaries, a temporary disruption of the cycle in 50% of rats immediately after exposure. These authors note that the most pronounced disruptions were observed in those cases in which tne irradiation was carried out during the oestrus. Genther (1934) also noted deviations in the course of the oestrous cycle immediately after a local Xray irradiation of guinea pigs; these disruptions were manifested by a prolongation of the first cycle.

In the work of Bischoff, Ulmann, and Ingraham (1944), data are presented which relate to the correlation between the extent of oestrous-cycle disruption in mice and the dosage of exposure. While on irradiation with 400r these authors observed in mice a complete supression of the oestrus immediately after exposure, on irradiation with 200r only a certain decrease

thereof was observed. Moreover, following irradiation with 400r the disruptions in the course of the oestrous cycle were of an irreversible nature (observations were continued for 13 1/2 months), whereas after an exposure to 200r, within 4 months the frequency of the oestrus in the irradiated animals did not differ from that of the controls.

As is apparent from the data cited, the material which has been made available hitherto does not permit reaching any well-defined conclusions concerning the regularities of the effects of ionizing radiation upon the oestrous cycle.

Material and Procedure

Sexually mature female mice of strain A, aged 2-3 months, weighing 18-25 g at the beginning of the experiments, were utilized as objects of the investigations.

Before and after irradiation the mice were kept in groups of six in 10-lit glass jars. In addition to the regular diet consisting of 2g bread, 5g milk, 5 g oats, and 0.1g hempseed or sunflower seed per mouse per day, the animals were periodically given chalk, and, during spring and summer, green fodder (dandelion and plantain leaves), and in the autumn and wintertime, carrots, fishliver oil, and yeast.

A total, single, Xray irradiation of the animals was carred out under the following conditions: voltage 160 kv; current intensity 5 ma; filters, 0.75 mm Al +0.5 mm cu; focal distance 40 cm; dose intensity 15.3 r/min. Dosages of exposure: 15, 25, 50, 100, 200, and 400r. The mice were irradiated in wooden boxes, 12 mice at a time. (For details of the irradiation procedure see the paper by N. I. Shapiro and N. I. Nuzhdin, "Effects of Different Dosages of Xray Irradiation on the Survival of Mice" which is included in the present symposium.)

Although in the case of most dosages, the irradiation was carried out in two or three repeated applications, we are presenting only the summative data. Each series of experiments had separate controls consisting of females of the same age which were not subjected to irradiation. The dosages of 200 r and 400r had common controls, since these experiments were carried out concurrently.

In investigating the oestrous cycle, use was made of vaginal smears. The freshly made unstained smear was examined at low magnification under the microscope. The samples were taken daily, except for nonworking days, during the first 3 months following exposure, and thereafter, following a 2-month interruption, over the entire sixth month.

In testing the irradiated females for fertility, the males were kept together with the females over a period of about 40 days. All instances of pregnancy and birth were recorded.

Investigation of the oestrous cycle by means of the method of vaginal smears, which permits forming an opinion concerning the functional state of the ovaries on the basis of the cell composition of the smear, is complicated by the fact that the cyclic process in mice is not strictly regular, even under normal conditions. Durations of the stages and cycle may show fluctuations not only as concerns different females, but even in the same animal. Moreover, the oestrous cycle, as any process, shows, in addition to well-defined principal stages, also intermediate stages which not infrequently can be determined only with knowledge of the preceding and the following stages. Taking all this into account, in order to make possible a comparison of the data, we have endeavored to unify the approach to the determination of the proportions of cells in the smear by means of repeated evaluations of the samplsamples by different persons, and to carry out the determination of the stages

according to a single pattern, viz., by using the following recording system. Plus signs were used to denote the presence of any given cells in the smear. One plus sign indicates the presence of a small number of cells, two plus signs indicate the presence of many cells, and three plus signs indicate the presence of very many cells. The basic type of cells in the proestrus were considered to be the epithelial cells; in the oestrus the squamae.

The metoestrus is characterized by the presence of epithelial cells and leukocytes in addition to the squamae; while in the dioestrus the principal type of cells are the leukocytes. To illustrate the recording system used by us we include herein a sample record (Table 1). Finally, controls were used in every case. This made it possible to compare in particular the data relating to experiments conducted at different times or by different persons, if not in absolute values characterizing the frequency of occurrence of any given stage of the genital cycle, then by means of indices (the ratios of data secured in the experiment is those obtained with the controls).

Finally, the objective nature of the conclusions arrived at was ensured by selection of a number of different indices characterizing the course of the genital cycle. These indices include the following: (1) frequency of the occurrence of individual stages of the oestrous cycle (in percent); (2) mean duration of the stages (in days); (3) number of females exhibiting the cyclic process (in percent); (4) mean number of normal cycles per female; (5) mean duration of the cycle (in days).

The Relationship Between Disruptions in the Course of the Oestrous Cycle and the Dosage of Exposure

To determine the relationship between disruption of the course of the oestrous cycle and the dosage of Xray exposure, a series of experiments

was carried out involving a single total Xray irradiation of the females with 15, 25, 50, 100, 200, and 400r.

As was stated hereinbefore, one of the characteristics of the oestrous cycle is the frequency of occurrence and mean duration of individual stages. Since in the normal cyclic process there is always observed a sufficiently definite sequence of stages, each of which has a definite duration, it is natural that disruptions of the oestrous cycle which occur in irradiated females must be reflected by these indices.

TABLE 1

Proestrus				Oestrus				Metoestrus				Dioestrus			
Epithelial cells	Squamae	Leukocytes	Mucus	Epithelial cells	Squamae	Leukocytes	Mucus	Epithelial cells	Squamae	Leukocytes	Mucus	Epithelial cells	Squamae	Leukocytes	Mucus
+++					+++			++	++	++		+		+++	+
++	++			+	+++			+	++	++		+	+	+++	
++	++	+						++	++	+++		++	+	+++	
	No cells								+++	+		+	-	+	

Table 2 shows the data on the frequency of occurrence and mean duration of individual stages of the oestrous cycle in animals at different time intervals following irradiation.

On examining the data of Table 2 we note first of all that, following exposure to 15 and 25r, the experimental animals and the controls show a similar distribution of the cycle stages. They are characterized by a high percentage of the occurrence of the stages of oestrus and dioestrus and a relatively lower frequency of the proestrous and metoestrous stages. The noted similarity is observed at all the time intervals of the investigation.

The least dosage of exposure among the ones that were investigated which affected the course of the oestrous cycle is the dosage of 50r. At this dosage, as well as the higher ones (100, 200 and 400r), the irradiated animals show a change in the distribution of the stages of the genital cycle. The degree of these changes depends directly upon the length of time elapsed since the irradiation and on the exposure dosage. During the first month after irradiation, with all the effective exposure dosages the percent ratio of stages in the experimental series approximates that in the controls. In the experimental animals during the second month after irradiation the frequency of the occurrence of individual stages is different from that found in the controls.. Conspicuous is the considerable decrease in the frequency of occurrence of the oestrous and proestrous stages and the corresponding increase in the frequency of metaoestrus and dioestrus.

TABLE 2

FREQUENCY OF OCCURRENCE AND DURATION OF INDIVIDUAL STAGES OF THE OESTROUS CYCLE IN IRRADIATED MICE

Exposure dosage (r)	Average number of females	Total number of tests	Dioestrus		Proestrus		Oestrus		Metoestrus	
			Number of tests (%)	Mean duration of stage (days)	Number of tests (%)	Mean duration of stage (days)	Number of tests (%)	Mean duration of stage (days)	Number of tests (%)	Mean duration of stage (days)
[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]
					First month					
25	24	624	34.7±1.91	2.5	16.2±1.47	1.3	32.7±1.88	2.1	16.4±1.47	1.5
Control	11	286	32.2±2.75	2.1	15.3±2.12	1.3	31.5±2.74	2.1	21.0±2.41	1.7
50	43	1150	39.2±1.44	3.4	10.3±0.89	1.3	33.8±1.40	2.8	16.7±1.12	1.8
Control	43	1171	44.5±1.46	3.6	8.9±0.84	1.3	29.0±1.33	2.4	17.6±1.12	1.9
100	24	600	27.0±1.81	2.2	13.3±1.38	1.3	46.0±2.04	2.9	13.7±1.41	1.4
Control	23	600	34.2±1.93	2.4	11.8±1.33	1.3	42.8±2.02	2.6	11.2±1.28	1.4
200	19	567	39.4±2.05	3.6	12.5±1.41	1.5	31.1±1.95	2.7	17.0±1.58	1.6
400	28	850	37.8±1.66	3.5	15.0±1.23	1.8	34.6±1.64	2.8	12.6±1.15	1.6
Control	17	514	40.8±2.16	3.7	10.5±1.38	1.3	33.5±2.08	2.7	15.2±1.57	1.5
					Second month					
25	24	600	34.2±1.93	2.3	16.5±1.50	1.2	34.6±1.95	2.2	14.7±1.46	1.4
Control	10	258	45.7±3.11	3.5	12.4±2.03	1.3	24.1±2.66	1.9	17.8±2.40	1.6

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]
50	43	1109	51.2±1.50	5.9	4.8±0.66	1.2	22.2±1.25	2.9	21.8±1.25	3.1
Control	43	1129	32.8±1.26	2.5	12.3±0.97	1.2	38.9±1.45	2.4	16.0±1.09	1.6
100	24	600	51.3±2.04	6.0	2.8±0.69	1.2	24.7±1.77	3.1	21.2±1.67	2.4
Control	23	600	29.0±1.85	2.0	13.3±1.38	1.4	44.5±2.03	2.6	13.2±1.37	1.4
200	18	551	53.8±2.12	6.6	2.2±0.62	1.3	20.1±1.70	3.2	23.9±1.82	2.4
400	25	735	55.8±1.84	5.8	2.7±0.63	1.3	11.2±1.15	2.2	30.3±1.69	2.9
Control	17	502	29.5±2.03	2.3	10.6±1.40	1.3	39.4±2.18	2.7	20.5±1.82	1.9
					Third month					
15	24	600	37.7±1.98	2.4	19.0±1.68	1.3	27.3±1.81	2.0	16.0±1.50	1.6
Control	23	587	38.8±2.02	2.4	16.1±1.51	1.3	27.9±1.86	2.0	17.2±1.52	1.6
25	23	592	36.7±1.99	2.3	13.8±1.43	1.3	37.2±1.99	2.2	12.3±1.34	1.2
Control	10	560	35.7±2.97	2.2	15.0±2.22	1.3	32.7±2.92	2.1	16.6±2.34	1.3
50	43	1063	53.3±1.53	8.4	1.1±0.31	1.2	4.9±0.67	2.0	40.7±1.51	6.7
Control	46	1150	23.0±1.24	2.2	10.4±0.91	1.2	40.3±1.45	2.5	26.3±1.29	1.8
100	24	600	68.1±1.90	7.4	1.7±0.57	1.4	7.0±1.05	2.2	23.2±1.72	2.9
Control	24	600	29.8±1.87	2.0	9.5±1.22	1.2	47.5±2.04	2.6	13.2±1.88	1.4
200	14	361	71.5±2.39	10.7	0.8±0.50	1.9	3.9±1.03	2.9	23.8±2.24	3.9
400	18	445	70.3±2.17	11.4	0.5±0.33	1.4	8.3±1.13	2.9	22.0±2.00	4.0
Control	13	335	33.4±2.57	3.2	9.5±1.64	1.4	35.6±2.62	2.9	21.5±2.27	2.4

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]
					Sixth month					
15	24	216	37.1±3.29	2.7	12.0±2.22	1.5	36.6±3.29	2.3	14.3±2.36	1.7
Control	23	207	36.2±3.35	2.3	17.4±2.62	1.6	33.4±3.27	2.3	13.0±2.34	1.5
25	20	500	42.8±2.22	3.0	13.0±1.50	1.3	23.4±1.88	1.9	20.8±1.82	2.0
Control	9	225	44.9±3.32	3.0	15.1±2.40	1.3	22.6±2.82	2.2	17.4±2.50	1.9
50	29	853	69.9±1.57	10.8	1.5±0.48	1.5	5.8±0.81	2.3	22.8±1.45	3.9
Control	32	924	35.0±1.57	2.3	17.0±1.24	1.4	31.4±1.52	2.1	16.6±1.24	1.5
100	14	370	67.8±2.43	10.7	0.5±0.36	1.1	13.0±1.76	5.0	18.7±2.04	3.8
Control	18	472	28.0±2.07	2.8	9.1±1.32	1.1	46.8±2.30	2.8	16.1±1.69	1.6
200	13	386	64.2±2.44	10.7	1.8±0.71	1.5	11.4±1.59	2.5	22.6±2.14	3.1
400	18	511	68.1±2.06	17.0	0.8±0.44	1.6	14.1±1.54	3.2	17.0±1.66	3.8
Control	13	380	40.3±2.51	3.5	9.7±1.54	1.5	33.9±2.44	3.2	16.1±1.38	2.0

As was pointed out before, the described changes are in a direct relationship to the exposure dosage. Thus, the percent of oestrus occurrence in the irradiated animals is lower in comparison with the controls by 1.7 times with 50r, 1.8 times with 100r, 2 times with 200r, and more than 3 times with 400r. Still more drastically reduced is the percent of occurrence of the proestrous stage. While after irradiation with 50r, the percent of tests showing the presence of proestrus is 2.5 times lower in the experiment than in the controls, after irradiation with 400r it is 4 times lower.

On irradiation with 400r, when a maximum decrease in the frequency of occurrence of the oestrus was observed, a conspicious fact is the increase in the percentage of the occurrence of metoestrus, with retention of about the same frequency of dioestrus as with the other effective dosages of exposure. Increase in the percent of metoestrus is due to the fact that during the period when disruptions of the oestrous cycle begin to manifest themselves (which with a dosage of 400r takes place during the second month following irradiation), although the occurrence of cycles is observed, they are in most cases atypical. The oestrous stage is encountered very rarely. As a rule it is replaced by the metroestrous stage. Occurrence of atypical oestrus was observed by Schubert (cited according to Zondek, 1938) upon the use of high dosages of Xray irradiation on mice ovaries. He determined that the cause of this phenomenon is a polymorphism of the vaginal epithelium. The different stages of the cycle can be seen, side by side, in the vagina of the irradiated animal on anatomical investigation. The vaginal smears taken at that time contain, in addition to squamae, also leukocytes and epithelial cells, and the stage assumes the form of metoestrus.

During the third month after irradiation of the females, with all the effective dosages of exposure, the percent of oestrus occurrence attains its minimum value, i.e., it is about equal with all the dosages. The percent of proestrus occurrence at that time is so low that this stage can be considered practically absent. Concurrently with decrease in frequency of the occurrence of oestrus and proestrus, there is observed a further increase in the frequency of occurrence of the dioestrous stage with all dosages except that of 50r. Only on exposure to 50r does the percent of dioestrus occurrence remain at its former level, there being observed a higher percentage than during the second month of the occurrence of metoestrus (a similar fact was noted by us with 400r during the second month following irradiation).

During the sixth month, the distribution of stages in irradiated animals is analogous to that observed during the third month. Dioestrus is encountered most frequently, then metoestrus, oestrus, and finally proestrus; and the proportion of the stages is about the same with all the effective dosages. It should be noted, however, that a certain increase takes place in the frequency of the oestrous stage occurrence during that period in comparison with the third month.

Analysis of data listed in Table 2 shows that disruptions of the cycle induced by Xray irradiation results in changes of the frequency of occurrence of individual stages of the oestrous cycle. Of special importance is the sharp decrease in the occurrence of the oestrus, a stage which is most indicative of the genital cycle, and the increased occurrence of dioestrus, a stage of relative rest, which indicates an absence of a cyclic process.

On taking into account the significance of the oestrous stage as an index of the presence of a genital cycle in the animals, we present graphs

(Figure 1) which depict the dynamics of the occurrence of this stage in animals previously subjected to exposure at different dosages of Xrays. The graphs provide a good illustration of the regularities observed in the changes of the frequency of occurrence of the oestrus at the different time intervals following irradiations which have been mentioned above.

Disruptions in the course of the oestrous cycle induced by irradiation are also reflected in the mean duration of the stages (see Table 2). With dosages of 15 and 25r, absence of disruptions in the cycle is confirmed by the good agreement of the values which characterize the mean duration of the different stages in the experiments and the controls (it should be mentioned that since tests were not carried out during the nonworking days, corrections have been made for the omitted days). During the first month following exposure to Xrays at a dosage of 50r and higher, the mean duration of all stages does not differ from the controls. The effects of irradiation at the stated dosages begin to manifest themselves only during the second month, at which time increased duration of the dioestrous stage is most apparent. With all dosages of exposure, the values characterizing the mean duration of this stage in irradiated animals are higher by more than 2 times than those relating to the control animals. During the third month there is noted a further increase in the duration of the dioestrous stage which exceeds by more than 3 times the duration of dioestrus in the controls.

During the sixth month after irradiation, the mean duration of dioestrus in the irradiated animals exceeds by 4 to 5 times the mean duration of dioestrus in the control animals. Thus, in females irradiated with 400r, the mean duration of dioestrus is of 17 days as compared with 3 1/2 days in the controls. A somewhat lesser increase takes place in the mean

duration of the metoestrous stage. The mean duration of proestrus and oestrus with all the dosages remains practically unchanged during all the time intervals of the investigation. Conspicuous is the fact that duration of the oestrous stage increased in females irradiated with 100r, in the sixth month after irradiation. This increase is the result of the fact that among the 14 mice which were under observation one was in the oestrous stage during the entire month.

The data which have been considered show that disruptions of the cycle are associated to a considerable extent with a change, mostly toward an increase, of the durations of the individual stages. Most characteristic is the occurrence in the animals of prolonged dioestrous periods; prolongation of the metoestrus is observed somewhat less often. In individual instances a prolonged oestrus is observed.

Although the frequency of occurrence of individual stages and their duration do reflect disruption of the course of the genital cycle in irradiated animals, it must be recalled that even in the presence of the oestrous stage and an alternation of the stages it is not certain that the cycle proceeds normally. The cycle is indeed an atlernation of stages occurring in a strictly defined sequence. Therefore, a more complete characterization of the oestrous cycle requires examination of the data showing the percentage of females undergoing the cycle, the average number of normal cycles per female, and the mean duration of the cycle (Table 3).

In processing the material upon which Table 3 is based we have made the following assumptions.

(a) A normal cycle is the combination of successively occurring stages of proestrus, oestrus, metoestrus, and dioestrus.

(b) A female undergoing cycles is a female in which at least one normal cycle was observed within the given interval of time. In computing the number of cycles per female and the duration of the cycle, the reckoning was always started at the stage which was observed in the female at the beginning of the experiment. Due to the fact that some stages are of such brief duration that they cannot always be recorded on single daily examination, we considered as a cycle not only those instances in which it was possible to consecutively record all the stages, but also those instances in which the cycle lacked some single stage (except the oestrus).

Data on the number of females undergoing cycle, shown in Table 3, indicate that with dosages of 15 and 25r the number of such females among the experimental animals does not differ from the number found among the controls. Larger dosages of irradiation result in a decrease of the number of females undergoing the cycle. This decrease depends upon the time interval following the irradiation, as well as on the dosage of exposure. Thus, during the first month after irradiation, the number of females undergoing the cycle shows no difference between the experimental and the control animals. During the second month the number of females undergoing the cycle decreases, this decrease being slight with a dosage of 50r, and the number of females undergoing the cycle being 90.9 percent as compared with 100.0 in the controls.

TABLE 3

EFFECTS OF DIFFERENT DOSAGES OF XRAY IRRADIATION ON THE COURSE OF THE OESTROUS CYCLE IN MICE

Time following irradiation	1st month				2nd month				3rd month				6th month			
Exposure dosage (r)	Total number of females	In cycle (%)	Average number of normal cycles per female	Mean duration of cycle (days)	Total number of females	In cycle (%)	Average number of normal cycles per female	Mean duration of cycles (days)	Total number of females	In cycle (%)	Average number of normal cycles per female	Mean duration of cycle (days)	Total number of females	In cycle (%)	Average number of normal cycles per female	Mean duration of cycle (days)
15	-	-	-	-	-	-	-	-	24	100.0	4.0± 0.45	6.3	-	-	-	-
Control	-	-	-	-	-	-	-	-	23	91.5	4.3±0.31	6.4	-	-	-	-
25	24	100.0	4.2±0.28	6.6	24	100.0	4.3±0.20	6.5	22	100.0	4.4±0.21	6.2	20	90.0	3.0±0.37	6.8
Control	11	100.0	4.0±0.44	6.0	10	90.0	2.9±0.60	6.5	10	100.0	4.2±0.39	6.1	9	100.0	3.2±0.49	8.5
50	46	93.5	3.0±0.20	7.9	44	90.9	1.7±0.15	9.3	41	34.4	0.4±0.14	12.7	35	22.9	0.4±0.16	13.4
Control	47	97.8	2.9±0.20	8.4	46	100.0	4.0±0.15	6.6	46	100.0	3.6±0.17	6.6	23	100.0	3.9±0.23	6.4
100	24	100.0	4.0±0.25	6.4	24	79.0	1.2±0.20	8.8	24	37.6	0.5±0.16	8.4	18	11.1	0.1±0.08	8.0
Control	24	95.6	4.2±0.29	6.2	24	95.6	4.2±0.28	6.1	24	100.0	4.3±0.27	5.7	23	95.5	2.4±0.24	6.9
200	22	95.4	2.8±0.35	8.9	22	59.0	0.6±0.13	11.5	15	6.7	0.1±0.06	6.0	16	37.5	0.4±0.16	11.2
400	30	93.3	2.5±0.26	7.9	30	30.3	0.6±0.16	10.9	19	10.5	0.1±0.07	11.8	22	13.7	0.2±0.14	7.2
Control	20	90.0	3.1±0.41	7.4	20	85.0	3.3±0.40	6.6	14	85.5	2.3±0.39	8.3	16	93.5	2.5±0.34	7.9

With a dosage of 100r, the percent of females in cycle decreases more, amounting to 79.0 as compared with 95.6 in the controls. With a dosage of 200r, it drops to 59.0, and with 400r it reaches the minimum value of 33.3, as compared with 85.0 in the controls. During the third month following irradiation the percentage of females in cycle decreases still further, amounting to 6.7-10.5 with the highest dosages. Finally, during the sixth month the percentage of females in cycle with all the dosages of exposure (except that of 200r) remains approximately at the same level as during the third month.

Figure 2 shows the curves which represent the changes in the number of females in cycle as a function of the exposure dosage and the time following the irradiation. Logarithms of the dosages are plotted along the axis of the abscissas, and along the axis of ordinates the indices representing the ratio of females in cycle in the experimental group to that in the controls, which is taken as being equal to 1. The curves show clearly that while during the first month the percent of females in cycle practically does not differ from that in the controls, at all the dosages of exposure, during the second month when disruptions in the cycle begin to manifest themselves there becomes apparent a well-defined correlation between extent of disruption in the cycle and the exposure dosage. With increasing dosage of irradiation, the number of females undergoing the normal cycle decreases. During the third month the number of females in cycle reaches its lowest level which is also retained during the sixth month following irradiation. The deviations which exist are within the limits of the errors and practically do not support any contention as to a restoration of the course of the oestrous cycle.

Most indicative is the characteristic of the irradiated animals according to the number of normal cycles per female. In animals irradiated

with 15 and 25r the average number of cycles during all the time intervals of the investigation, per one female does not differ from that found in the controls. At higher dosages the effects of irradiation also become manifest as concerns this index only one month after the exposure. In this instance the minimum effective dosage of Xrays is also one of 50r. The extent of decrease in the number of cycles per female is directly correlated with the dosage of exposure. While during the second month following irradiation with 50r the number of cycles per female is 1.7, as compared with 4.0 in the controls, after 100r it is 1.4 in lieu of 4.2 in the controls; and after irradiation with 200 and 400r, the number of cycles per female is 0.6 as compared with 3.3 in the controls. Thus, during the second month following irradiation the females subjected to 200 and 400r are practically no longer undergoing the cycle. During the third month after Xray irradiation with a dosage of 50r, decrease in cycling reaches the same level as that observed during the second month in the case of the application of a 400r dosage of Xrays, i.e., on the average the females have not undergone a single complete cycle. Females irradiated with 200 and 400r show only 0.1 in lieu of 2.3 cycles found in the controls. Analysis of the data relating to the sixth month fails to reveal any restoration of the cycle.

Figure 3 shows the curves which illustrate the correlation between the mean number of cycles per female and the exposure dosage at different time intervals during the investigation. As in the foregoing graph, the logarithms of dosages are plotted along the axis of abscissas; the axis of ordinates shows the indices (ratios of average number of cycles per female in the experiments and the controls). The curves show clearly that in the irradiated animals the average number of cycles per female is inversely correlated with the dosage of exposure and the time interval following irradiation.

The data listed in Table 3, concerning the mean duration of the cycle in irradiated and control animals, show that in females subjected to the action of 15 and 25r the mean duration of the cycle during all the time intervals of the observations virtually does not differ from those of the controls. Upon use of higher dosages, during the first month after irradiation a great similarity is also found to exist between the experimental and the control data. Beginning with the second month, there is apparent in almost all instances a tendency toward an increase of the mean duration of the cycle in the irradiated animals. Reaching any kind of definite conclusion concerning the mean duration of the cycle during the later time intervals of the investigation does not appear possible, since the number of normal cycles in the irradiated mice is sharply decreased at that time. In any event, no specially drastic deviations in the duration of individual cycles have been noted.

Thus the data which we have obtained show in a sufficiently convincing manner that the ionizing radiation exercises a strong influence upon the course of the oestrous cycle. According to this test, the minimum effective dosage of a single Xray irradiation was found to be 50r. Disruptions of the cycle in females treated with this dosage of Xray manifest themselves not immediately, but only after the lapse of about a month and a half following irradiation. By that time the irradiated females exhibit arrhythmicalness of the cyclic process which manifests itself in changes of the duration of the stages of the genital cycle as well as in a disruption of their usual sequence. Most characteristic is the steadily decreasing frequency of the occurrence of the oestrous stage which in a number of instances is replaced by the metoestrous stage whereby the cycle becomes atypical. Following longer time intervals after irradiation the alternation of stages occurs steadily less regularly, and normal cycles become rarer. During the sixth month following irradiation most females show prolonged periods

of metoestrus or dioestrus which indicates an actual cessation of cycle, and only in individual females are isolated instances of normal cycle occasionally encountered.

Upon application of higher dosages of Xrays (100, 200, 400r) duration of the latent period is somewhat reduced, and already at the beginning of the second month there is noted an appreciable inhibition of the oestrous cycle. At the same time, the greater the dosage of exposure, the sharper the manifestations of the disruptions. By the third month after irradiation (50-400r), the cyclic process practically ceases in all the investigated females. The data obtained also support the statement that, with exposure dosages of 50-400r, disruptions of the course of the oestrous cycle are of an irreversible nature within the time intervals studied in the present investigation. On taking into account the facts that Xray irradiation with 50r has practically no sterilizing effect on the males and produces only a slight, rapidly subsiding effect upon the organism as a whole, the conclusion can be reached that dissruption of the oestrous cycle constitutes a very sensitive index of the damaging action of the radiation.

Effects of Small Dosages of Xrays on the Fecundity of Females

As was noted hereinbefore, the course of the oestrous cycle constitutes a good, yet indirect, indication of the decreased fertility of females. Therefore, it was important to ascertain how fertile are the females which had been subjected to Xray exposure but did not exhibit disruptions of the oestrous cycle. In this connection, of considerable interest was the study of such exposure dosages at which the females do not show any particular deviations in the course of the oestrous cycle. Of importance was also an investigation of the fecundity of females subjected to an exposure to a threshold dosage (50r). In such a case, because of the

different biological sensitivity of the animals, there are also found, in addition to females in which disruptions of the oestrous cycle are observed as a result of the irradiation. Some females which show no deviations in the course of the oestrous cycle, are also of interest in connection with the study of the effects of Xrays on fecundity.

We have studied the fertility of females irradiated with 15, 25, and 50r. As was shown hereinbefore, Xray irradiation with 15 and 25r induces no appreciable change in the oestrous cycle of mice; whereas a dosage of 50r is a threshold dosage, following which disruptions of the oestrous cycle are observed in most females.

As to their fertility, only those females were tested which did did not show disruptions of the oestrous cycle. For 2 weeks prior to mating, the oestrous cycle of the females was investigated. Thereafter, the females in which the oestrous cycle was normal following irradiation, were mated with nonirradiated males. Analysis of the fertility was carried out at different time intervals sufficiently remote from the time of irradiation. After 3 1/2 months the fertility was tested in females irradiated with 25 and 50r, and after 5 1/2 months in those which had been exposed to 15 and 25r.

Table 4 shows the data characterizing the fertility of females 3 1/2 months after irradiation.

TABLE 4

FERTILITY OF FEMALE MICE EXPOSED TO SMALL DOSAGES OF XRAYS

Exposure dosage (r)	Tested females in cycle	Produced offspring		Number of litters	Number of offspring		Average number of offspring per litter	% of stillbirths
		Number	Percent		Total	Stillborn		
25	42	20	47.7±7.7	18	112	1	6.2±0.45	0.9±0.9
50	16	4	25.0±11.2	4	13	1	3.3±0.76	7.7±7.7
Control	45	36	80.0±5.9	32	227	9	7.1±0.32	4.0±1.3

The figures listed in the table show first of all that the absence of disturbances of the oestrous cycle in females is not always an indication of their normal fertility. This is shown by the lower percentage of females which produced offspring among those which had a normal oestrous cycle. In the irradiated series the number of females which produced offspring is lower than in the controls. Moreover the figures indicate very well a direct correlation between reduced fertility and exposure dosage. Whereas with a dosage of 25r the number of females which produced offspring is about 1 1/2 times lower than in the controls (the difference is reliable: $M_{dif} = 32.3 \pm 9.8$), irradiation with 50r resulted in a decrease of the number of females that produced offspring by more than 3 times in comparison with the controls. The difference in this instance is also reliable ($M_{dif} = 55.0 \pm 1.27$).

Exposure to small dosages of Xrays results not only in a decrease of the number of litters produced by the females but also in a decrease of the number of offspring per litter. Even with a dosage of 25r the size of the litter decreases in comparison with the controls (Table 4). Apparently it is only the insufficient amount of material relating to this group of irradiated females that constitutes the explanation of the unreliability of the difference between experiment and control. In females irradiated with 50r the average number of offspring per litter decreases still further, amounting to 3.2 mice in lieu of the 7.1 mice in the controls. In this instance the difference is reliable ($M_{dif} = 3.9 \pm 0.8$).

Noteworthy is also the certain increase in the number of stillbirths in the litters of the irradiated females (50r). But in this respect the relatively scant material does not permit speaking of a statistical reliability of the increase in stillbirths in the case of females which had been subjected to irradiation.

Investigation of the fertility of females 5 1/2 months after irradiation also shows that the extent of reduction in the fertility of the animals following irradiation depends on the exposure dosage. Thus, with a dosage of 15r, 58.2% of the females produced offspring (7 out of 12 mice), in lieu of 83.2% (10 of 12 mice) in the controls. With a dosage of 25r, none of 8 irradiated females produced any offspring, whereas in the controls the number of females which produced offspring reached 50% (4 out of 8 mice). A comparison of the data relating to the fertility of the females tested at different time intervals following irradiation supports the statement that decrease in fertility depends not only on the exposure dosage but also on the time which has elapsed thereafter. For example, while with a dosage of 25r, after 3 1/2 months following irradiation, the number of females which produced offspring reached 25.0%, after 5 1/2 months it dropped to zero. It is true that in the controls there was observed, after this time interval, a certain decrease in the number of females which produced offspring, due apparently to age-induced changes. Still, this decrease was considerably less than in the experiment. Data on the number of offspring in litters of females irradiated with 15r show that 5 1/2 months after irradiation the number of offspring in experiment and control are the same (the average size of the litters of females irradiated with 15r is of 5.3 mice, and of 5.5 mice in the controls.)

Thus, the data obtained show that even such a relatively small dosage of Xrays as 15r has a negative effect on the fertility of the females. While in this case the irradiation results only in a decrease of the number of females capable of producing offspring after an irradiation with 25r, there is added thereto a decrease in the number of offspring per litter. With 50r, the two above-mentioned characteristics are found in combination with a third, viz., an increased number of stillbirths.

It must be emphasized once again that the described phenomena, which show the negative influence of Xrays on fertility of female mice, were elicited in females which revealed no deviation in the course of the oestrous cycle due to exposure to Xrays. Consquently, notwithstanding the fact that the oestrous cycle is highly radiosensitive, the fertility of female mice -- in the precise meaning of this term -- is still more sensitive to the action of Xrays.

Effects of Xray Irradiation on the Oestrous Cycle of Mice of Different Strains, and of Mice in Different Physiological Conditions

Data showing the high radiosensitivity of female mice as concerns their fertility were obtained with animals of strain A which had never previously given birth. Since every strain of mice is characterized by different physiological features, it is not excluded that the radiosensitivity which we have ascertained is characteristic not of mice in general, but only of that strain which was used in the experiments. In order to determine to what extent the regularities which have been revealed are inherent to different strains of mice, additional experiments were undertaken. Thus the effects of Xray irradiation were studied not only on females of strain A, but also on females of strain C_{57} (black). These two strains differ from each other in a number of biochemical, physiological (particularly in their hormonal level), and morphological indices.

Of no lesser importance was a determination of the extent the radiosensitivity of the females in relation to their fertility is dependent upon their physiological state. In particular it was of interest to ascertain whether or not animals which had previously propagated and those which had not react in the same manner to Xray irradiation. For this purpose we have carried out special experiments in which a comparative evaluation was made of the radiosensitivity of females appertaining to different strains, on

the one hand, and on the other of females which had or had not previously given birth. To provide a solution of the first formulated problem, young, sexually mature, nonparent females of strain C_{57} (black) (24 animals) were subjected to a single total irradiation with a dosage of 100r. The condition of irradiation were the same as those utilized with animals of strain A. The controls consisted of 25 females of strain C_{57} (black), of the same age.

Results of the experiments on Xray irradiation of females of strain C_{57} (black), were compared with analogous data obtained with mice of strain A. Table 5 shows the percent ratios and duration of the individual stages of the oestrous cycle in the mice of the strains under investigation. These data testify to the similarity of the disruptions of the oestrous cycle in irradiated mice of strain A and C_{57} (black). Experimental groups of mice of toth strains show, beginning with the second month following irradiation, an appreciable decrease in the frequency of occurrence of the stages of oestrus and proestrus, and increased frequency of the occurrence of the dioestrous and metoestrous stages. A still more considerable decrease in the frequency of occurrence of the stages of oestrus and proestrus was observed during the third and sixth month following irradiation. For example, during the third month, in the females of strain A, 8.7% of the total number of tests indicated a stage of oestrus and proestrus; while in females of strain C_{57} (black) this figure was 10%, as compared with 57 and 50% in the controls. During the sixth month the data were 13.5 and 7.55% respectively for the experimental animals; 55.9 and 45.9% for the controls. It should also be noted that with increasing disruption of the oestrous cycle in the irradiated mice there is observed a prolongation of the stages of dioestrus and metoestrus, and this is more sharply manifested in females of strain A. The mean duration of the stages of oestrus and proestrus in experimental and control animals showed no difference. Drastic prolongation of the oestrous stage in females of strain A during the sixth month following irradiation is connected with a long-lasting oestrus in one of the females, which also constitutes an indication of the disruption of the genital cycle in the irradiated mice.

TABLE 5

FREQUENCY OF OCCURRENCE AND DURATION OF THE INDIVIDUAL STAGES OF THE OESTROUS CYCLE IN IRRADIATED MICE OF DIFFERENT STRAINS (DOSAGE OF XRAY IRRADIATION 100r)

Month Following Irradiation		1				2			
Strain		A		C_{57} (Black)		A		C_{57} (black)	
		Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Average number of females		24	24	24	25	24	24	20	25
Total number of tests		600	600	624	650	600	600	511	642
Dioestrus	Number of tests (%)	27.0±1.82	34.2±1.93	32.1±1.87	29.4±1.75	51.3±2.23	29.0±1.89	40.6±2.17	38.5±1.91
	Mean duration of stage (days)	2.16	2.40	2.59	2.20	6.00	2.03	3.08	2.73
Proestrus	Number of tests (%)	13.3±1.37	11.8±1.32	12.5±1.34	13.7±1.36	2.8±0.76	13.3±1.38	4.8±0.96	10.7±1.23
	Mean duration of stage (days)	1.31	1.43	1.27	1.23	1.20	1.43	1.31	1.24
Oestrus	Number of tests (%)	46.0±2.04	42.8±2.02	27.8±1.86	34.1±1.81	24.7±1.77	44.5±2.03	20.1±1.77	33.0±1.85
	Mean duration of stage (days)	2.88	2.64	2.08	2.09	3.12	2.64	2.20	2.26
Metoestrus	Number of tests (%)	13.7±1.43	11.2±1.28	27.6±1.86	25.5±1.38	21.2±1.66	13.2±1.37	34.5±2.10	17.8±1.28
	Mean duration of stage (days)	1.43	1.43	2.32	2.03	2.40	1.43	2.76	1.88
		3				6			
Average number of females		24	24	16	21	14	18	16	17
Total number of tests		600	600	400	525	370	472	400	425
Dioestrus	Number of tests (%)	68.1±1.91	29.8±1.87	26.3±2.2	36.0±2.10	67.8±2.42	28.0±2.06	51.5±2.5	37.9±2.35
	Mean duration of stage (days)	7.40	2.03	2.44	2.20	10.60	2.76	5.88	3.02
Proestrus	Number of tests (%)	1.7±0.57	9.5±1.22	3.0±0.85	15.6±1.60	0.5±0.36	9.1±1.31	3.3±0.89	16.2±1.78
	Mean duration of stage (days)	1.44	1.20	1.20	1.20	1.14	1.20	1.29	1.47
Oestrus	Number of tests (%)	7.0±1.04	47.5±2.03	7.0±1.27	34.4±2.06	13.0±1.75	46.8±2.29	4.3±1.01	29.2±2.2
	Mean duration of stage (days	2.16	2.64	1.52	2.10	5.03	2.76	2.55	2.13
Metoestrus	Number of tests (%)	23.2±1.72	13.2±1.37	63.7±2.41	14.0±1.51	18.7±2.04	16.1±1.68	40.9±2.46	16.7±1.82
	Mean duration of stage (days)	2.88	1.43	4.44	1.61	3.78	1.60	4.19	1.85

Figure 4 shows the curves which characterize the frequency of occurrence of individual stages of the oestrous cycle in mice of strain A and C_{57} (black). Frequency of occurrence of the oestrous stage (and also of the other stages) is expressed on the curves in indices which indicate the ratio of the percent of irradiated females in the oestrous stage to the percent of such females in the controls. Expressed in an analogous manner are the other data pertaining to the course of the oestrous cycle. The curves show clearly the same nature of the changes of the oestrous cycle in the irradiated females of both strains under study.

The similarity noted becomes even more apparent on comparison of the data on the number of normal cycles per female and the number of females in cycle. From Table 6 which shows these data it is apparent that disruptions in the course of the oestrous cycle in females of both strains begin with the second month following irradiation and during the third and sixth months the cyclic process of the females ceases almost completely. While in the control groups, during the entire period of investigation, there occur 3.5 to 4 normal cycles monthly per female, with a mean duration of the cycle being 6-7 days; in the irradiated mice, on the second month following exposure, there occur 1.2 cycle per female of strain A and 2.3 cycle per female of strain C_{57} (black). On the other hand, during the third and sixth months after irradiation, there are respectively 0.5 and 0.6 cycle per female, with a mean duration of the cycle being about 8 days. The difference between experiment and control as concerns females of both strains is statistically fully reliable. Exceptions are the data relating to the second month after irradiation in the case of mice of the strain C_{57} (black), wherein the reliability of the difference is equal to 2.56. Similar data were also obtained on the percent of females in cycle.

For a more apparent comparison of the course of the oestrous cycle in mice of strains A and C_{57} (black), the above-stated data are presented in the form of curves in Figures 5 and 6. In this instance, as before, the average number of cycles per female and the percent of females in cycle are expressed in indices (the ratio of experiment to control).

The results of the experiments permit reaching the following conclusion. Disruptions of the oestrous cycle, induced by a single total Xray irradiation with a dosage of 100r, are of a similar nature in mice of strains A and C_{57} (black). Thus, the latent period in both strains lasts approximately one month, and disruption of the normal cyclic process of females is observed only beginning with the second month following irradiation. During the third and sixth month after irradiation there is observed an almost complete cessation of the cyclic process.

To determine the question concerning the nature of the reaction to Xray irradiation in parent and nonparent females, a special experiment was carried out. Upon reaching sexual maturity, a group of females of strain A, of the same age, was kept with males of the same strain. Of this group of mice 73 females were selected, which had given birth twice in succession (without having suckled the offspring), of which 24 were subjected to a single total Xray irradiation dosage of 50r, 22 to a dosage of 100r, the 27 nonirradiated females serving as controls. In the enumerated females the oestrous cycle was studied according to the procedure adopted by us. The results of this experiment are compared with the data obtained on single total Xray irradiation (with the same dosages) of nonparent females of strain A.

Tables 7 and 8 show the percent proportions and the duration of individual stages of the oestrous cycle in parent and nonparent females. The data of Table 7 show that with a dosage of 50r disruptions in the course of the oestrous cycle in parent and nonparent females, begin with the second month following irradiation. In the nonparent females the difference

between experiment and control in the percent of occurrence of individual stages of the oestrous cycle is statistically reliable, whereas in the case of the parent females these differences -- although they do not reach a statistical reliability -- are of sufficiently high probability (reliability of the difference as concerns the oestrus stage is 2.28; it is 2.46 as concerns the proestrous stage). In the nature of disruptions of the course of the oestrous cycle during the third and sixth months following irradiation, the groups of females being compared show no differences. It is possible that the later manifestation of disruptions in the course of the oestrous cycle in the parent females as compared with the nonparent constitutes an indication of their somewhat enhanced resistance to Xray irradiation. However, no definite conclusion can be reached on the basis of the data cited.

A comparison of the results concerning the Xray irradiation of parent and nonparent females with a dosage of 100r (Table 8) shows that disruptions in the course of the oestrous cycle in the animals of both groups occur similarly. In the parent, as well as in the nonparent females, beginning with the second month after irradiation, there is observed a statistically reliable -- as compared with the controls -- lowering of the percent of occurrence of the stages of oestrus and proestrus, and an increase in the stages of dioestrus and metoestrus. During the third and sixth months the differences between experiment and control are manifested in animals of both groups even more sharply.

For a more apparent comparison of the frequency of occurrence of individual stages of the oestrous cycle in parent and nonparent females the corresponding data are expressed in indices (ratios of experiment data to the control data taken as equal to 1), and are represented as curves in Figure 7.

TABLE 6

EFFECTS OF XRAY IRRADIATION ON THE COURSE OF THE OESTROUS CYCLE IN MICE OF DIFFERENT STRAINS. (IRRADIATION DOSAGE 100r)

Time following irradiation	1 month				2 months			
Strain	A		C_{57} (black)		A		C_{57} (black)	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Total number of females	24	24	24	25	24	24	16	24
Percent of females in cycle	100.0	95.6	96.0	96.0	79.0	95.6	81.5	100.0
Average number of normal cycles per female	4.0±0.25	4.2±0.29	3.3±0.31	3.76±0.33	1.2±0.20	4.2±0.28	2.31±0.41	3.54±0.26
Mean duration of normal cycle (days)	6.4	6.2	6.73	6.52	8.8	6.1	6.77	6.31
	3 months				6 months			
Total number of females	24	24	16	21	18	23	16	17
Percent of females in cycle	37.5	100.0	43.7	95.2	11.1	95.5	25.0	100.0
Average number of normal cycles per female	0.5±0.16	4.3±0.27	0.63±0.22	4.05±0.28	0.1±0.03*	2.4±0.24*	0.5±0.28	3.64±0.24
Mean duration of normal cycle (days)	8.4	5.7	5.52	5.62	8.0	6.9	8.17	7.48

*Oestrous cycle examined during course of 3 weeks.

TABLE 7

FREQUENCY OF OCCURRENCE AND DURATION OF INDIVIDUAL STAGES OF THE OESTROUS CYCLE IN BRED AND UNBRED FEMALES OF STRAIN A ON SINGLE XRAY IRRADIATION (DOSAGE 50r)

Time following irradiation		1 month				2 months			
		Bred		Unbred		Bred		Unbred	
	Females	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Number of females		24	27	43	43	24	25	42	43
Total number of tests		600	656	1150	1171	600	626	1109	1129
Dioestrus	Number of tests (%)	40.7±2.00	35.8±1.87	39.2±1.44	44.5±1.46	47.7±2.04	38.2±1.9	51.2±1.5	32.8±1.26
	Mean duration of stage (days)	2.74	2.28	3.4	3.6	3.6	2.28	5.9	2.5
Prooestrus	Number of tests (%)	15.6±1.48	17.2±1.47	10.3±0.89	8.9±0.84	10.5±1.31	15.3±1.44	4.8±0.66	12.3±0.97
	Mean duration of stage (days)	1.31	1.35	1.3	1.3	1.3	1.39	1.2	1.2
Oestrus	Number of tests (%)	29.7±1.86	28.1±1.75	33.8±1.40	29.0±1.33	23.2±1.72	28.9±1.81	22.2±1.25	38.9±1.45
	Mean duration of stage (days)	1.99	1.78	2.8	2.4	2.21	1.92	2.9	2.4
Metoestrus	Number of tests (%)	14.0±1.41	18.9±1.52	16.7±1.12	17.6±1.12	18.6±1.58	17.6±1.52	21.8±1.25	16.0±1.09
	Mean duration of stage (days)	1.38	1.54	1.8	1.9	1.94	1.63	3.1	1.6
		3 months				6 months			
Number of females		24	24	43	43	19	17	29	32
Total number of tests		586	588	1063	1150	459	414	853	924
Dioestrus	Number of tests (%)	53.6±2.06	35.4±1.97	53.3±1.53	23.0±1.24	70.2±2.13	48.7±2.45	69.9±1.57	35.0±1.57
	Mean duration of stage (days)	5.26	2.17	8.4	2.2	10.5	3.5	10.8	2.3
Oestrus Prooestrus	Number of tests (%)	6.0±0.98	15.2±1.48	1.1±0.31	10.4±0.91	4.8±0.99	12.3±1.61	1.5±0.48	17.0±1.24
	Mean duration of stage (days)	1.33	1.33	1.2	1.2	1.6	1.3	1.5	1.4
	Number of tests (%)	12.2±1.35	27.4±1.84	4.9±0.67	40.3±1.45	7.6±1.04	20.2±1.97	5.8±0.81	31.4±1.52
	Mean duration of stage (days)	2.14	1.85	2.0	2.5	2.56	1.77	2.3	2.1
Metoestrus	Number of tests (%)	28.2±1.86	22.0±1.7	40.7±1.51	26.3±1.29	17.4±1.77	18.8±1.92	22.8±1.45	16.6±1.24
	Mean duration of stage (days)	2.93	2.08	6.7	1.8	3.6	1.62	3.9	1.5

TABLE 8

FREQUENCY OF OCCURRENCE AND DURATION OF INDIVIDUAL STAGES OF THE OESTROUS CYCLE IN PARENT AND NONPARENT FEMALES OF STRAIN A ON SINGLE XRAY IRRADIATION (DOSAGE 100r)

Time following irradiation		1 month				2 months			
Females		Bred		Unbred		Bred		Unbred	
		Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Number of females		22	20	24	24	22	19	24	24
Total number of tests		550	490	600	600	550	473	600	600
dioestrus	Number of tests (%)	46.0±2.12	33.7±2.13	27.0±1.82	34.2±1.93	56.4±2.11	39.2±2.23	51.3±2.23	29.0±1.89
	Mean duration of stage (days)	3.45	1.94	2.16	2.4	6.6	2.32	6.0	2.03
prooestrus	Number of tests (%)	14.2±1.48	16.9±1.69	13.3±1.37	11.8±1.32	5.8±0.99	16.4±1.69	2.8±0.70	13.3±1.37
	Mean duration of stage (days)	1.46	1.36	1.31	1.43	1.33	1.46	1.2	1.43
oestrus	Number of tests (%)	24.6±1.83	28.2±2.04	46.0±2.04	48.2±2.02	11.4±1.35	26.8±2.03	24.7±1.77	44.5±2.03
	Mean duration of stage (days)	2.16	1.82	2.88	2.64	1.92	1.9	3.12	2.64
metoestrus	Number of tests (%)	15.2±1.53	21.2±1.84	13.7±1.43	11.2±1.28	26.4±1.88	17.6±1.74	21.2±1.66	13.2±1.3
	Mean duration of stage (days)	1.44	1.57	1.43	1.43	3.17	1.53	2.4	1.43
		3 months				6 months			
Number of females		22	18	24	24	13	12	14	18
Total number of tests		533	450	600	600	307	292	370	472
dioestrus	Number of tests (%)	66.0±2.05	35.2±2.25	68.1±1.91	29.8±1.87	86.1±1.97	48.0±2.92	67.8±2.42	28.0±2.06
	Mean duration of stage (days)	8.45	2.23	7.4	2.03	14.3	3.83	10.6	2.76
prooestrus	Number of tests (%)	2.6±0.68	15.7±1.71	1.7±0.57	9.5±1.22	1.3±0.64	13.0±1.97	0.5±0.36	9.1±1.31
	Mean duration of stage (days)	1.40	1.29	1.44	1.20	1.53	1.27	1.14	1.14
oestrus	Number of tests (%)	3.2±0.76	24.4±2.02	7.0±1.04	47.5±2.03	0.6±0.43	22.2±2.43	13.0±1.75	46.8±2.29
	Mean duration of stage (days)	2.91	1.73	2.16	2.64	1.15	1.83	5.03	2.76
metoestrus	Number of tests (%)	28.2±1.95	24.7±2.03	23.2±1.72	13.2±1.37	12.0±1.85	16.8±2.18	18.7±2.04	16.1±1.68
	Mean duration of stage (days)	4.09	2.25	2.88	1.43	3.88	1.55	3.78	1.6

It is apparent from the curves that during the first month after irradiation no substantial difference in oestrous-cycle indices are observed between the parent and nonparent animals. The changes setting in during the second month proceed in both groups in a sufficiently similar manner.

Tables 9 and 10 show data on the percentage of females in cycle and the number of normal cycles per female in the groups of animals being compared. On comparison of these indices parent and nonparent mice show a similar picture of disruptions induced by irradiation with dosages of 50 and 100r, as compared with the corresponding control groups. While in the control animals, the number of normal cycles per female fluctuates from three to four per month; in the experimental females irradiated with 50r (Table 9), during the second month after exposure, the number of cycles in parent females is 2.67, and in the nonparent it is 1.7. During the third month it is respectively 1.22 and 0.4; during the sixth 0.59 and 0.4 cycle. In animals irradiated with a dosage of 100r (Table 10) during the second month after irradiation we find 1.59 cycles per female among the parent females and 1.2 cycles among the nonparent females, During the third month we have, respectively, 0.1 and 0.5 cycle; during the sixth, 0 and 0.1.

Figure 8 shows the curves which represent the number of normal cycles per female for the parent and nonparent mice, which had been irradiated with dosages of 50 and 100r. The curves show clearly that the disruptions in the cyclic process of parent and nonparent females are similar.

As concerns the percentage of females in cycle (Figure 9), there is noted a regularity similar to that which was observed in the number of normal cycles per female.

There should also be noted the certain tendency toward an increase in the duration of the cycle in animals of the experimental group with progressing disruption of their cyclic process.

TABLE 9

EFFECTS OF XRAY IRRADIATION ON THE COURSE OF THE OESTROUS CYCLE IN PARENT AND NONPARENT MICE OF STRAIN A (IRRADIATION DOSAGE 50r)

Time following irradiation	1 month				2 months			
	Parent		Nonparent		Parent		Nonparent	
Females	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Number of females	24	26	40	47	24	25	44	46
Number of females in cycle (%)	95.6	96.0	93.5	97.8	95.6	91.8	90.9	100.0
Average number of normal cycles per female	3.92±0.29	4.07±0.29	3.0±0.20	2.90±0.26	2.67±0.32	4.2±0.34	1.7±0.15	4.0±0.15
Mean duration of normal cycle	5.83	6.03	7.9	8.4	6.9	5.7	9.3	6.6
	3 months				6 months			
Number of females	24	24	41	46	17	15	35	28
Number of females in cycle (%)	62.4	95.6	24.4	100.0	58.7	86.7	22.9	100.0
Average number of normal cycles per female	1.22±0.26	4.09±0.34	0.4±0.14	3.6±0.17	0.59±0.26	2.86±0.46	0.4±0.16	3.9±0.23
Mean duration of normal cycle	8.65	6.03	12.7	6.6	14.0	6.2	13.4	6.4

TABLE 10

EFFECTS OF XRAY IRRADIATION ON THE COURSE OF THE OESTROUS CYCLE IN BRED AND UNBRED MICE OF STRAIN A. (IRRADIATION DOSAGE 100r)

Time following irradiation	1 month				2 months			
Females	Parent		Nonparent		Parent		Nonparent	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Number of females	22	20	24	24	22	19	24	
Number of females in cycle (%)	91.0	85.0	100.0	95.6	81.7	89.5	79.0	95.6
Average number of normal cycles per female	3.26±0.34	4.0±0.35	4.0±0.25	4.2±0.29	1.59±0.25	3.94±0.41	1.2±0.20	4.2±0.28
Mean duration of normal cycle	7.05	5.94	6.4	6.2	7.9	5.82	8.8	6.1
	3 months				6 months			
Number of females	20	18	24	24	11	10	18	23
Number of females in cycle (%)	10.0	94.5	37.6	100.0	0	90.0	11.1	95.5
Average number of normal cycles per female	0.1±0.67	3.8±0.42	0.5±0.16	4.3±0.27	0	3.0±0.58	0.1±0.08*	2.4±0.24*
Mean duration of normal cycles	4.2	6.27	8.4	5.7	0	6.8	8.0	6.9

*Oestrous cycle examined during 20 days

The results of experiments on the study of the radiosensitivity of parent and nonparent mice support the opinion that disruptions of the oestrous cycle, induced by a single total Xray irradiation with dosages of 50 and 100r, progress very similarly in animals of both groups. In the parent as well as in the nonparent females irradiated with 100r, there is observed, beginning with the second month, an equally sharp disruption of the normal cyclic process, which virtually ceases altogether by the third and sixth month.

A similar nature of changes in the course of the oestrous cycle is also noted in parent and nonparent mice irradiated with a dosage of 50r. At the same time in the parent females, during the second month after irradiation, the differences in the cyclic process between experimental and control animals are manifested less sharply than in the nonparent females.

On summarizing the analysis of all the data presented in the present section of this paper, the following basic conclusion can be reached. The high radiosensitivity of nonparent females as concerns disruptions of the oestrous cycle, which has been revealed on investigation of animals of strain A, is also inherent in other strains of mice. Evidently it is to a considerable extent independent of certain physiological conditions of the animals which have been subjected to the exposure.

Effects of Chronic Gamma Irradiation on the Oestrous Cycle

After data had been secured showing the great reactivity of female mice, as concerns their fertility, to a single Xray irradiation, it was decided to determine the nature of the effect on the course of the oestrous cycle of chronic exposures, especially at dosages approximating the tolerated. A resolution of this question is not only of unquestionable theoretical interest, but to a certain extent also of a

practical interest. At the same time, we note that to our knowledge there has been only one investigation concerned with the study of the effects of chronic exposure to gamma radiations on the course of the oestrous cycle (Levitin, 1940).

Co^{60} was utilized as the source of radiation. Study of the effects of chronic gamma irradiation on the oestrous cycle was conducted on female mice of strain C_{57} (black). Co^{60} as the source of gamma radiation, was kept in a special container provided with an automatic elevator and a clockwork mechanism. Every day the container was automatically lifted out of a pipe set in the floor of the room in which the mice were located, and after 8 hours it was lowered back into the pipe. Thus, the irradiation of the animals was effected for 8 hours daily except during nonworking days.

The mice were kept in wooden cages which were disposed in a circle on four racks located at different distances from the source of irradiation. During the 8 hours of irradiation, the mice located on the first rack were exposed to a dosage equal on the average to 0.4r; those on the second rack to 0.2r; those on the third to 0.1r; and those on the fourth rack to 0.05r.

(Design of the container which held the Co^{60}, as well as of the dosimetry, was done by Professor Ya. L. Shekhtman, for which the authors wish to express their profound gratitude).

In the experiments use was made of sexually mature mice weighing 18-22g. Altogether two series of experiments were carried out. Animals of the first series were subjected to the action of gamma rays during 18 months, and were placed on the first and fourth rack. The second series was started 3 months later than the first. The animals of the second series were irradiated during 15 months and were placed on the

second, third, and, in part, on the fourth rack. Mice located on the first, second, third, and fourth rack will be referred to in the following description as animals on the first, second, third, and fourth group.

As controls, use was made of nonirradiated females of the same age and of the same strain -- C_{57} (black). Due to a number of circumstances, the conditions under which the mice of the experimental series were kept differed somewhat from those of the controls. Thus, the mice undergoing irradiation were kept throughout the duration of the experiments in a heated basement room under artificial light. The animals of the control series were kept in an unheated room during spring, summer, and autumn, and were transferred to a heated room only when cold weather set in. Feeding conditions of experimental and control animals were the same.

During the progress of the experiments, the irradiated and control females were investigated as to the oestrous cycle 4 times over a period from 20 to 30 days each. The oestrous cycle of the females of the first series was examined on the fourth, eighth, twelfth, and eighteenth month after beginning of the irradiation; and those of the second series during the first, fourth, eighth, and fifteenth month.

The summative dosages of exposure at the beginning and the end of the oestrous-cycle examinations are shown in Table 11. Mice of the first and second group received a relatively larger summative dose of irradiation, which attained, by the end of the experiments, 186.5r in the first group and 77.4r in the second group. Mice of the third and fourth group received a summative dose of irradiation of less than 50r, i.e., less than the dosage which, on single exposure, can be considered to a certain extent as a threshold dosage, as was shown in the previously-described experiments.

Table 12 shows data on the percent ratio and duration of individual stages of the oestrous cycle in mice of the experimental groups during different periods of irradiation.

The data of Table 12 show the occurrence of age-induced changes of the oestrous cycle in experimental as well as in the control mice, which are manifested by a decrease with age of the percentage of the occurrence of the oestrus stage with a corresponding increase of the dioestrous and metoestrous stages. However, even with this background of age-induced changes 12 and 18 months after the beginning of irradiation there is observed an almost statistically-reliable decrease of the percent of oestrus occurrence in females of the first group as compared with the controls. Reliability of the difference in the former instance (with a summative dosage of 118.7r) is 2.24, and in the latter (with a summative dosage of 178r) 2.77. The lower percentage of oestrus occurrence in comparison with the control was also observed in the second group of females (irradiated with a daily dosage of 0.2r) on the fifteenth month after the beginning of the experiment (summative dosage of irradiation equal to 72.8-77.4r). It is true that in this instance the difference is not statistically reliable (1.37). In the third group of females, with a daily dosage of irradiation equal to 0.14, regular differences from the controls could not be detected. A certain decrease in the frequency of oestrus occurrence, observed in the experimental mice during the eighth month, is of a temporary nature, the frequency of the oestrus reverting thereafter to the normal during the fifteenth month. In both series of mice of the fourth group, which received a daily dosage of irradiation equal to 0.05r, the percent of oestrus occurrence even exceeds in some instances that of the controls.

TABLE 11

DOSAGES OF γ-IRRADIATION ACCUMULATED AT THE TIME OF EXAMINATION OF THE OESTROUS CYCLE

No of series	Group of animals	Daily irradiation dosage (r)	1st-Examination: Number of months since beginning of irradiation	1st-Examination: Irradiation dosage (r) At beginning of examination	1st-Examination: Irradiation dosage (r) At end of examination	2nd-Examination: Number of months since beginning of irradiation	2nd-Examination: Irradiation dosage (r) At beginning of examination	2nd-Examination: Irradiation dosage (r) At end of examination	3rd-Examination: Number of months since beginning of irradiation	3rd-Examination: Irradiation dosage (r) At beginning of examination	3rd-Examination: Irradiation dosage (r) At the end of examination	4th-Examination: Number of months since beginning of irradiation	4th-Examination: Irradiation dosage in (r) At beginning of examination	4th-Examination: Irradiation dosage in (r) At end of examination
1	1	0.4	4	36	43.2	8	74.9	81.8	12	118.7	127.8	18	178	186.5
	4	0.05	4	4.5	5.4	8	9.35	10.2	12	14.85	16.0	18	22.1	23.35
2	2	0.2	1	2	5.6	4	21.4	24.8	8	43.0	48.0	15	72.8	77.4
	3	0.1	-	-	-	4	10.7	12.4	8	21.7	24.0	15	36.4	38.7
	4	0.05	1	0.5	1.4	4	5.35	6.2	8	10.7	12.0	15	18.2	19.35

Concerning the duration of individual stages it may be noted that on chronic exposure, as on single irradiation, the disruptions of the oestrous cycle involve increase of the duration of the stages of dioestrus and metoestrus with more or less equal duration of the oestrous and proestrous stages.

For a more complete disclosure of the disturbances of the oestrous cycle in irradiated mice, Table 13 includes data relating to the percent of females in cycle, number of normal cycles per female, and the duration of the normal cycle. These indices, together with determinations of the relative frequency of the individual stages of the cycle, sufficiently reflect the course of the oestrous cycle in the irradiated and the control females.

On analysis of the numerical data which characterize the number of normal cycles per female, it is necessary to bear in mind that during the third and fourth examinations the duration of the observations was extended to 26 to 30 days, in lieu of 20 to 22 days in the first two examinations. Hence, a comparison of the changes of this index in the irradiated and control mice can be made only for each examination separately.

On comparing the data concerning the number of normal cycles per female in the irradiated and the control animals, it is noted that in the first group of females irradiated with a daily dosage of 0.4r during the twelfth and eighteenth month after the beginning of the experiment there is found a certain decrease of this index, viz., 1.95 cycle in the experimental, as compared with 2.62 cycles in the control animals during the twelfth month, and 0.52 cycle and 1.0 cycle during the eighteenth. Analogous changes are also found in the percentage of females in cycle: 78.5 during the twelfth month, and 53.8 during the eighteenth, in the irradiated animals; and 87.5 and 62.5 respectively in the control animals. The appreciable decrease of the number of normal cycles and also of the percentage of females in cycle in the control group of mice of the first series during the eighteenth

month after the beginning of the experiment can be attributed to the occurrence of age-induced changes. This cannot be said of the mice of the control group of the second series included in the investigations 3 months later. During the fifteenth month after the beginning of the experiment all the females are in cycle. A more substantial decrease of the number of normal cycles per female as compared with the controls is observed during the fifteenth month after the beginning of irradiation in mice of the second and third group. While in the second group, during the thirty day period of investigation, there is 1.26 normal cycle, and 1.05 in the third; in the control group of mice of the same age there are found 2.16 normal cycles per female during the same period of investigation. Reliability of the difference in that instance between experimental and control animals, in the second group was 2.18, and 3.27 in the third. In these groups of mice, during the fifteenth month after the beginning of irradiation, there is also observed a lower percentage of females in cycle in comparison with the control. While in the controls all the females were in cycle during this period, among the mice of the second group their number amounted to 73.6%, and in the third group 79%.

The fourth group of mice of the first series shows equal or somewhat higher -- as compared with the controls -- indices of the normal cycles per female and percentage of females in cycle. In the females of the fourth group of the second series during the first two periods (first and fourth month after beginning of the experiment), there is noted a fairly substantial -- in comparison with the controls -- excess in the number of normal cycles per female. During the two subsequent periods (eighth and fifteenth months) it is replaced by a slight, statistically-unreliable decrease of this index. The percentage of females in cycle does not show such deviations.

TABLE 12

FREQUENCY OF OCCURRENCE AND DURATION OF THE OESTROUS-CYCLE STAGES IN MICE OF THE STRAIN C_{57} (BLACK) ON CHRONIC GAMMA IRRADIATION

No of series						1					
Groups of animals		1				4			Control		
Daily dosage of irradiation (r)		0.4				0.05					
Summative dosage of irradiation at start of oestrous-cycle examination (r)	36.0	74.9	118.7	178.0	4.5	9.35	14.85	22.1	-	-	-
Number of months after beginning of experiment	4	8	12	18	4	8	12	18	4	8	12
Number of females	23	24	24	15	15	16	16	13	16	16	16
Total number of tests	413	431	561	332	270	266	382	325	288	271	380
Dioestrus — Number of tests (%)	52.5±2.46	46.9±2.4	56.1±2.1	53.6±2.74	40.7±2.99	50.3±3.06	53.5±2.55	41.5±2.74	51.4±2.95	56±3.01	59.1±2.52
Dioestrus — Duration of stage (days)	3.6	3.24	4.9	7.6	2.8	3.33	3.9	5.4	3.93	3.9	4.6
Proestrus — Number of tests (%)	4.6±0.84	6.0±1.14	6.0±1.0	0.9±0.57	2.6±0.96	5.3±1.37	6.3±1.24	1.2±0.6	5.2±1.31	3.7±0.14	6.3±1.24
Proestrus — Duration of stage (days)	1.45	1.16	1.16	1.19	1.22	1.36	1.16	1.6	1.3	1.18	1.24
Oestrus — Number of tests (%)	39.5±2.41	32.9±2.27	16.3±1.55	9.9±1.63	51.1±3.04	35.8±2.94	26.3±2.25	26.5±2.45	38.2±2.86	32.9±2.85	22.2±2.13
Oestrus — Duration of stage (days)	2.36	2.27	1.77	2.62	3.12	2.38	4.06	3.32	2.8	2.7	2.0
Metoestrus — Number of tests (%)	3.4±1.02	14.2±1.57	21.6±1.75	35.6±2.64	5.55±1.39	8.6±1.72	13.9±1.76	30.8±2.56	5.2±1.31	7.4±1.59	12.4±1.69
Metoestrus — Duration of stage (days)	1.42	1.87	3.16	5.02	1.4	1.29	1.83	3.04	1.4	1.69	1.67

TABLE 12 (continued)

[a]	Number of series					2						
[b]	Groups of animals		2				3				4	
[c]	Daily dosage of irradiation (r)		0.2				0.1				0.05	
[d]	Summative dosage of irradiation at start of oestrous-cycle examination (r)	-	2.0	21.4	43.0	72.8	10.7	21.7	36.4	0.5	5.35	10.7	18.2
[e]	Number of months after beginning of experiment	18	1	4	8	15	4	8	15	1	4	8	15
[f]	Number of females	9	24	24	24	19	24	24	18	8	8	8	5
[g]	Total number of tests	210	432	408	570	475	408	574	450	144	134	189	124
[h]	Dioestrus: Number of tests (%)	47.1±3.43	36.5±2.32	43.6±2.45	48.2±2.09	22.6±1.92	42.6±2.44	50.3±2.09	22.3±1.96	40.3±4.07	43.3±4.26	45.5±3.62	24.2±3.83
[i]	Dioestrus: Duration of stage (days)	7.0	2.44	2.82	3.9	3.13	2.62	3.51	3.87	2.74	3.12	4.58	5.15
[j]	Prooestrus: Number of tests (%)	0.9±0.65	3.5±0.88	5.6±1.13	7.7±1.11	0.4±0.28	5.2±1.09	6.1±1.0	1.3±0.53	2.1±1.19	1.5±1.05	7.9±1.96	2.4±1.37
[k]	Prooestrus: Duration of stage (days)	1.19	1.30	1.17	1.22	2.4	1.17	1.32	1.2	1.22	1.18	1.46	1.2
[l]	Oestrus: Number of tests (%)	18.6±2.68	54.5±2.39	42.2±2.44	27.7±1.87	28.0±2.06	41.7±2.44	24.6±1.79	36.9±2.28	52.8±4.16	43.3±4.26	24.4±3.12	42.8±4.44
[m]	Oestrus: Duration of stage (days)	2.76	3.1	2.46	2.12	2.34	2.57	1.96	3.7	2.81	2.29	2.14	3.03
[n]	Metoestrus: Number of tests (%)	33.4±3.25	5.5±1.09	8.6±1.38	16.3±1.54	49.0±2.29	10.5±1.51	19.0±1.64	39.5±2.31	4.8±1.77	11.9±2.79	22.2±3.01	30.6±4.13
[o]	Metoestrus: Duration of stage (days)	3.34	1.53	1.28	2.13	3.98	1.4	1.71	3.5	1.7	1.56	2.46	2.16

[a]			2		
[b]			Control		
[c]			-		
[d]		-	-	-	-
[e]		1	4	8	15
[f]		9	8	7	6
[g]		161	135	168	150
[h]		46.0±3.92	44.5±4.28	58.4±3.79	28.0±3.66
[i]		3.8	3.17	3.82	2.97
[j]		4.9±1.67	2.9±1.44	6.5±1.89	2.6±1.29
[k]		1.22	1.18	1.16	1.2
[l]		39.8±3.84	40.0±4.2	28.6±3.48	34.0±3.86
[m]		3.01	2.54	2.07	3.05
[n]		9.3±2.29	12.6±2.85	6.5±1.89	35.4±3.9
[o]		1.41	1.53	1.28	2.54

TABLE 13

OESTROUS CYCLE IN MICE OF STRAIN C_{57} (BLACK) ON CHRONIC GAMMA IRRADIATION*

[1]	Number of series	1											
[2]	Groups of animals	1				4							
[3]	Daily dosage of irradiation (r)	0.4				0.05							
[4]	Summative dosage of irradiation at start of oestrous cycle examination (r)	36.0	74.9	118.7	178.0	4.5	9.35	14.85	22.1	-	-	-	
[5]	Number of months after beginning of experiment	4	8	12	18	4	8	12	18	4	8	12	
[6]	Number of females	23	24	23	13	15	15	16	13	16	16	16	
[7]	Percent of cycling females	100.0	100.0	78.5	53.8	100.0	100.0	100.0	77	100.0	100.0	87.5	
[8]	Number of days of examination per one mouse	22	21	28	30	22	20	28	30	22	20	28	
[9]	Average number of normal cycles per female	2.8±0.19	2.5±0.19	1.95±0.28	0.61±0.22	2.53±0.23	2.26±0.21	2.56±0.24	1.38±0.33	2.31±0.25	2.0±0.15	2.62±0.34	
[10]	Mean duration of normal cycle (days)	6.4	7.5	6.88	11.5	6.7	6.60	7.26	10.6	8.2	7.2	7.15	

[1]	2														
[2]	2			3			4					Control			
[3]	0.2			0.1			0.05					-			
[4]	-	2.0	21.4	43.0	72.8	10.7	21.7	36.4	0.5	5.35	10.7	18.2	-	-	-
[5]	18	1	4	8	15	4	8	15	1	4	8	15	1	4	8
[6]	8	24	24	24	19	24	24	18	8	8	8	5	9	8	7
[7]	62.5	96.0	100.0	96.0	73.6	96.0	96.0	79.0	100.0	100.0	85.6	100.0	100.0	87.5	85.7
[8]	30	22	20	28	30	20	28	30	22	20	28	30	22	20	28
[9]	1.0±0.32	2.74±0.28	2.4±0.13	2.7±0.26	1.26±0.28	2.5±0.19	2.62±0.28	1.05±0.17	3.25±0.31	2.25±0.25	2.12±0.55	1.8±0.37	2.33±0.23	1.87±0.44	3.29±0.71
[10]	11.6	6.5	6.47	7.12	10.0	7.1	7.55	11.9	5.9	5.33	7.3	9.1	8.35	8.2	6.98

Control (continued)

-

15

6

100.0

30

2.16±0.30

8.4

Concerning changes in the duration of a normal cycle it should be pointed out that only in the cases of manifest disruption of the cyclic process of the females is there observed an increase in the duration of the cycle lasting approximately 6 to 8 days to 11 to 12 days.

For better illustration, the differences between experimental and control groups of animals in the percent of occurrence of the oestrus and the number of normal cycles per female are represented graphically in Figures 10 and 11. Changes in these characteristics are also expressed as indices (experiment/control). The graphs clearly reveal the general trend toward a decrease of the percentage of oestrus occurrence and the number of normal cycles per female in the first, second, and third group of animals, with increasing length of the observation period and the increasing summative dosage of exposure to gamma rays, and also a somewhat more intensive -- as compared with the controls -- cyclic process in the females of the fourth group of the first experimental series. The certain difference in the nature of the curves relating to the fourth group of the second series can apparently be attributed to the small number of animals investigated in this series. Increased percentage of the occurrence of oestrus in mice of the third group, in comparison with the controls, during the fifteenth month after the beginning of irradiation, with a statistically reliable decrease of the number of normal cycles during the same period of investigation, is explained by the occurrence in many mice of this group of a prolonged oestrus on disruption of the sequence of alteration of the cycle stages. This phenomenon must be unquestionably regarded as one of the manifestations of a disruption in the course of the oestrous cycle.

As was mentioned before, in the conduct of our experiments it was not possible to ensure identical conditions in the keeping of the experimental and control mice. On taking into account this circumstance and also the fact that in the mice of the fourth group of both experimental series no

inhibiting action of irradiation on the oestrous cycle could be detected, and that they remained at all times under the same conditions as all the other experimental mice, we considered it necessary to carry out a comparison of data secured in the first three groups with those relating to the fourth group which was considered conventionally as control.

Figures 12 and 13 show the curves which represent the frequency of occurrence of the oestrus and the number of normal cycles per female in mice of first, second, and third group. These characteristics are expressed in indices wherein the unit of comparison are the characteristics relating to the fourth group. On comparison of data on the percentage of occurrence of the oestrus, we find a statistically fully-reliable decrease of this characteristic in the first group of females during the fourth, twelfth, and eighteenth month (with a reliability of the difference amounting, respectively, to 3, 3.67, and 5.66), and in the second group during the fifteenth month after the beginning of the experiment (reliability of the difference is of 3.04). In mice of the third group, the percentage of the oestrus occurrence is also lower than in the mice of the fourth group, but the difference is not statistically reliable.

Changes in the number of normal cycles per female, as is apparent from Figures 12 and 13, proceed analogously to the changes in the percentage of oestrus occurrence. However, apparently due to the small number of animals under observation, these differences were found to be not entirely reliable statistically. In the first group of females, in comparison with the fourth, the reliability of the difference relating to the characteristic under consideration, during the twelfth month is 1.64, and during the eighteenth 1.9; while in the second and third group, during the fifteenth month, in relation to the corresponding data of the fourth group, it is 1.17 and 1.83.

Thus, the results of a comparison of the characteristics of the course of the oestrous cycle in animals of first, second, and third group with the fourth were found to be quite similar to those which were obtained when nonirradiated mice were taken as the standard of comparison.

At the same time, it is unquestionable that the nature of changes of the oestrous cycle in females of the first three experimental groups has become much more evident upon their comparison with the fourth experimental group in lieu of the controls.

On summing up the data presented in this section, we can state with certainty that female mice are highly radiosensitive as concerns their fertility, not only on single Xray irradiation, but also on chronic gamma irradiation with dosages approximating the tolerated. Thus, for example, according to our data, substantial disruptions of the oestrous cycle are observed in mice of the first group during the twelfth month, when the summative dosage of exposure reaches 118r. However, bearing in mind that these disruptions become manifest only after a certain latent period following the irradiation, equal on the average to 1 1/2 to 2 months, it can be considered that on chronic exposure about 90-100r are sufficient to induce disruption of the course of oestrous cycle in mice. On considering that disruptions in the fertility of the females occur most likely before changes take place in the course of the oestrous cycle, it can be assumed that on chronic irradiation danger is involved with summative dosages considerably lower than 90-100r.

Conclusions

1. Single total Xray irradiation of female mice results in a disruption of the course of the oestrous cycle. Decrease takes place in the number of females in cycle, as well as in the average number of cycles

per female. Disruptions of the cyclic process manifest themselves in decreased frequency of the occurrence of the proestrous and oestrous stages and a corresponding increase of the stages of the metoestrus and dioestrus.

2. The extent of disruption of the oestrous cycle is in a direct relationship to the dosage of exposure and time interval following the irradiation.

3. The oestrous cycle of female mice is most radiosensitive. The minimum effective dosage of a single Xray irradiation is about 50r.

4. Disruption of the cyclic process in females irradiated with 50r occurs not immediately, but only after 1 1/2 months. Arrythmicalness increases gradually and by the third month the cyclic process practically ceases. On exposure to larger dosages (100, 200, and 400r) duration of the latent period is somewhat decreased, and already at the beginning of the second month there is observed an appreciable inhibition of the oestrous cycle. By the third month following irradiation the cyclic process ceases in all the investigated females.

5. Within the limits of a 16-month period following Xray irradiation with dosages of 50-400r, disruptions of the oestrous cycle in female mice are of an irreversible nature.

6. A single, total Xray irradiation with dosages of 15 and 25r does not affect the course of the oestrous cycle of female mice, but at the same time decreases their fertility.

7. Disurption of the oestrous cycle in mice induced by a single total Xray irradiation is of a similar nature: (a) in female mice of strains A and C_{57} (black) in tests over 6 months following irradiation with 100r; (b) in parent and nonparent females of strain A in tests over 6 months following irradiation with 50 and 100r.

8. Chronic exposure to small doses of gamma rays also induces disruptions of the course of the oestrous cycle in female mice of strain C_{57} (black).

(a) Beginning with the twelfth month following irradiation with a daily exposure dosage of 0.4r (summative dosage of 118.7r),

(b) During the fifteenth month after beginning of irradiation with a daily exposure dosage of 0.2r (summative dosage of 72.8r) and 0.1r (summative dosage of 36.4r), there is a tendency toward a depression, in comparison with the controls, of the frequency of occurrence of the oestrus and of the number of normal cycles per female.

(c) With a daily exposure dosage of 0.05r no changes were observed in the course of the oestrous cycle.

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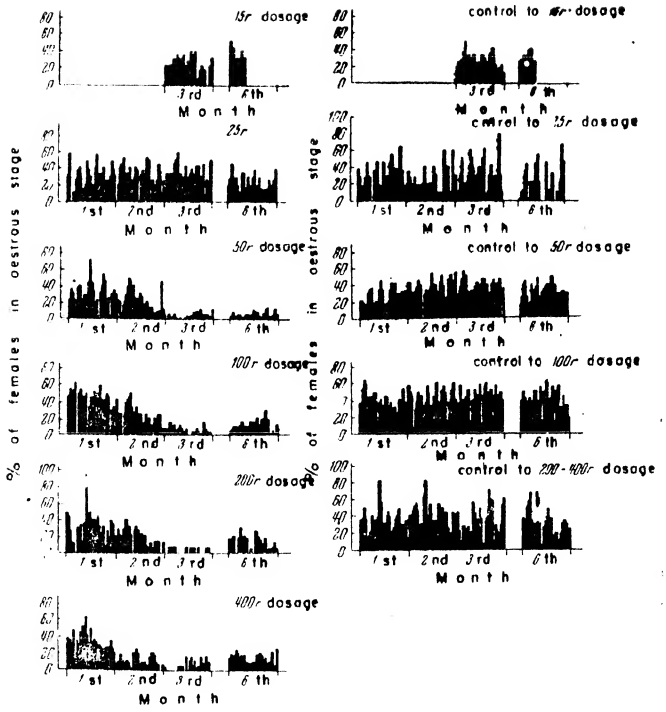

Figure 1. Occurrence of the oestrous stage in mice previously exposed to different dosages of X rays.

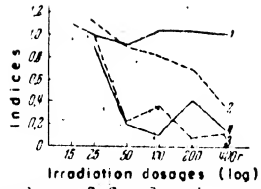

Figure 2. Changes in number of females in cycle, depending on the exposure dosage.

Time following irradiation: 1, first month; 2, second month; 3, third month; 4, sixth month.

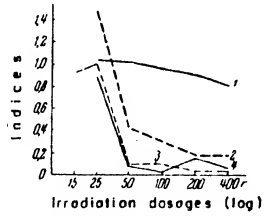

Figure 3. Changes in average number of cycles per female, depending on exposure dosage.

Time following irradiation: 1, first month; 2, second month; 3, third month; 4, sixth month.

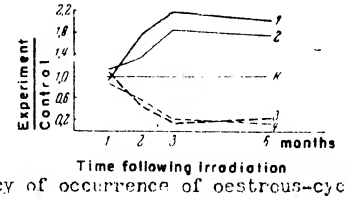

Figure 4. Frequency of occurrence of oestrous-cycle stages in irradiated mice of strain A and C_{57} black (in indices, experiment / control).

Dioestrus and metoestrus: 1, mice of strain A; 2, mice of strain C_{57}.

Oestrus and proestrus: 3, mice of strain A; 4, mice of strain C_{57}; k - control.

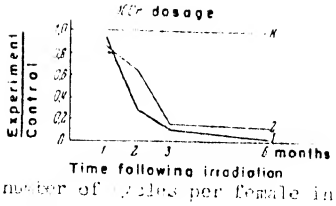

Figure 5. Average number of cycles per female in irradiated mice of strain A and C_{57} black (in indices experiment / control):
1, females of strain A; 2, females of strain C_{57}; k, control.

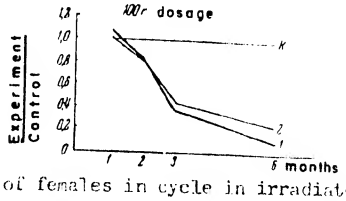

Figure 6. Percent of females in cycle in irradiated mice of strain A and C_{57} black (in indices experiment / control):
1, females of strain A; 2, females of strain C_{57}; k, control.

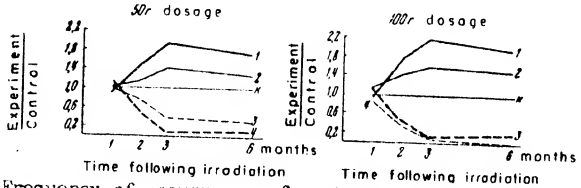

Figure 7. Frequency of occurrence of oestrous cycle stages in irradiated parent and nonparent females of strain A (in indices experiment / control).
Dioestrus and metoestrus: 1, nonparent females; 2, parent females. Oestrus and proestrus: 3, nonparent females; 4, parent females; k, control.

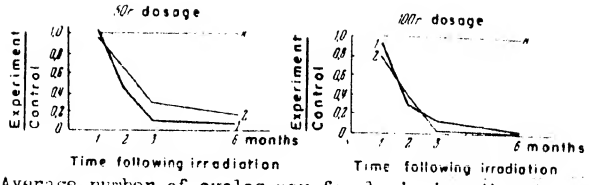

Figure 8. Average number of cycles per female in irradiated parent and nonparent females of strain A (in indices experiment / control):

1, nonparent females; 2, parent females; k, control.

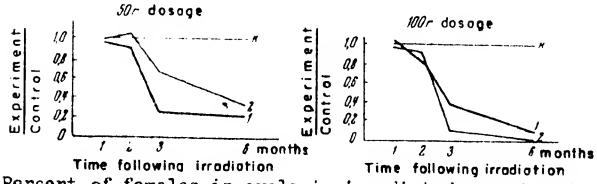

Figure 9. Percent of females in cycle in irradiated parent and nonparent mice of strain A (in indices experiment / control):

1, nonparent females; 2, parent females; k, control.

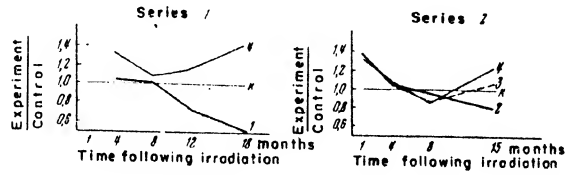

Figure 10. Frequency of occurrence of oestrus in mice on chronic gamma-irradiation (in indices experiment / control):

1, first group 0.4 r; 2, second group 0.2 r; 3, third group 0.1 r; 4, fourth group 0.05 r; k, control.

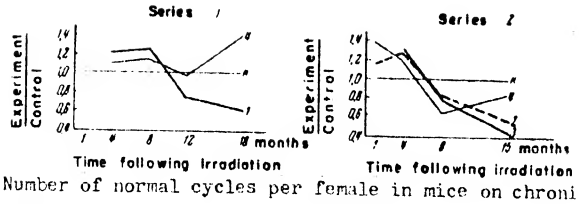

Figure 11. Number of normal cycles per female in mice on chronic gamma-irradiation (in indices experiment / control): 1, first group 0.4 r; 2, second group 0.2 r; 3, third group 0.1 r; 4, fourth group 0.05 r; k, control.

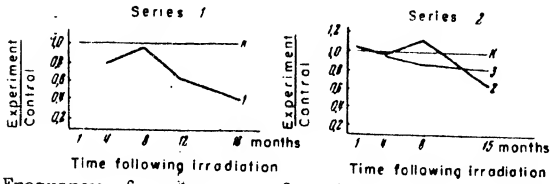

Figure 12. Frequency of occurrence of oestrus in mice on chronic gamma-irradiation (the fourth group is taken as controls): 1, first group 0.4 r; 2, second group 0.2 r; 3, third group 0.1 r; k, control (fourth group).

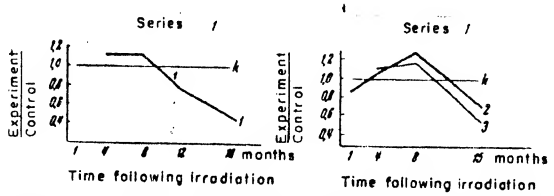

Figure 13. Number of normal cycles per female in mice on chronic gamma- irradiation (the fourth group is taken as controls): 1, first group 0.4 r; 2, second group 0.2 r; 3, third group 0.1 r; k, control (fourth group).

STERILIZING ACTION OF IONIZING RADIATION ON MAMMALS

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COMMUNICATION III

HEREDITARY NATURE OF STERILITY INDUCED BY THE ACTION OF XRAY IRRADIATION

In the preceding two communications data were presented relating to the effects of Xray irradiation on the fertility of mice subjected to direct exposure and also on the development of their offspring. It has been shown that Xrays possess strong sterilizing action as concerns females as well as males. To a considerable extent the mechanism of this action has been elucidated, and the nature of fertility-decrease has been ascertained, depending on the exposure dosage and the different time intervals following the irradiation. It was ascertained that sterility brought about by ionizing radiation is complex in its nature and comprises qualitatively different phenomena. Thus, in the irradiated males decreased fertility occurs in the presence of: (a) reduced copulatory capability; (b) damage to sperms without loss of their fertilization capacity, which causes the death of a considerable portion of the offspring during different stages of embryogenesis; (c) sterility as such arising essentially due to disruption of spermatogenesis which leads to cessation of the normal replenishment of the reserves of sperms.

After the lapse of a certain length of time following irradiation (which depends upon the exposure dosage) the males show a recuperation of fertility. The investigation has revealed that offspring sired after this recuperation (i.e. derived from genital cells which at the time of irradiation were in the stage of spermatogonia) bear no gross marks of the damaging action of radiation. In females, in contrast to the males, we

have not observed a recuperation of fertility after their sterilization by Xrays.

After ascertaining such a detailed picture of the sterilizing action of radiation on animals having been subjected directly to the irradiation, it was natural to consider the question as to whether or not the reproductive capability is also impaired in subsequent generations. This was the more necessary, in as much as at the present time data have accumulated in the literature indicating the hereditary nature of the sterilizing action of Xrays. In most investigations conducted on this subject, the objects of studies were either plants or invertebrates (Drosophila, Habrobackon, Bombyx, etc). Only in a few contributions was this question studied on mammals (mice) (Snell 1933; Russell, 1952). In these investigations it was ascertained that sterility of the offspring derived from irradiated parents is connected primarily with the occurrence in the genital cells of the latter of chromosome aberrations of the translocation type (Snell, Bodemann, and Hollander, 1934).

As a result, offspring derived from genital cells bearing the translocation is of strongly decreased fertility (the so-called semisteriles). In addition to translocations, sterility of the first and subsequent generations can be caused by other hereditary changes induced by the action of radiation (Neuhaus, 1937; Berg, 1938).

Since, as we have pointed out hereinbefore, data on inheritance of sterility in mammals induced by Xray irradiation are very scant, we deemed it necessary to continue our investigations in this field. We considered it of interest also for the reason that in the previously-published work the authors have studied the sterilizing action of local rather than total irradiation. Moreover, such an investigation would make it possible, under conditions of a single type experimental procedure,

to compare directly the sterilizing action of Xray irradiation with that which is observed in the offspring of specimens which had been subjected to the exposure, and also with the general nature of radiation reaction arising in animals following total Xray irradiation.

The present investigation is thus concerned with a study of the fertility of male mice derived from the mating of nonirradiated females with irradiated males.

As objects of the investigation use was made of sexually-mature, 2- to 3-month-old male mice of strain A derived from the mating of irradiated males (analyzed in the paper of N. I. Nuzhdin, N. I. Shapiro, and O. N. Petrova, included in the present symposium) with nonirradiated females.

In some cases the offspring tested were sired immediately after an Xray irradiation of the males (the first two litters), whereas in others they were sired 3 months after the exposure (sixth and seventh litters). The initially-utilized males were irradiated with dosages of 200r or 400r. The conditions of irradiation were as follows. Voltage 160 kv; current intensity 5 ma; filters 0.7 mm Al ± 0.5 mm Cu; focal distance 40 cm; dosage intensity 15.3r/min. Every variant of the experiments was provided with controls of the same age (males of strain A, derived from nonirradiated parents). Experimental and control animals were kept under identical conditions. Fertility of the tested males of the first generation was evaluated on the basis of the results of their mating with normal females of strain A.

Testing of the fertility of the first-generation males was effected by keeping each of them together with two sexually-mature females. After 14 days, these females were removed and replaced by others. In this manner every male was kept together, successively, with 6-8 females. After being separated from the males, the females were kept separately. All

instances of birth were recorded, noting the number of living and stillborn young in the litter. Determination of the sex was done on young mice 28 days of age. In addition thereto, there was also investigated the postembryonic development of the animals of the second generation.

The experiments included four experimental groups of first generation males, which differed on the one hand by the exposure dosage to which their sire was subjected (200-400r), and on the other by the time interval elapsed since this exposure (immediately after irradiation and 3 months thereafter). In addition to these groups of mice, there were also two control groups of animals, born from nonirradiated parents at points of time corresponding to those of the experimental groups of offspring. Consequently, the fertility was studied on six groups of males.

In the present work the following indices were utilized to characterize the fertility: procreative capability of the males (i.e. the fact of siring offspring), the number of effective matings (i.e. the number of copulation of males and females which resulted in birth of offspring), size of the resulting litters, and the number of stillborn young in the litters. A genetic analysis was also made of individual males of the first generation which exhibited reduced fertility.

In the course of the investigation it was ascertained that the first-generation males of the experimental series do not differ from the controls either in mating capability or number of effective matings with the females. Data on the mating capability of first generation males are shown in Table 1.

TABLE 1

MATING CAPABILITY OF FIRST GENERATION MALES

Irradiation dosage applied to the initial animals (r)	Time of Siring of First Generation								
	Immediately after irradiation (litters 1 and 2)			3 months after irradiation (litters 6 and 7)			Total		
	Males tested	Sired offspring		Males tested	Sired offspring		Males tested	Sired offspring	
		Number	Percent		Number	Percent		Number	Percent
200	23	22	95.6±4.3	8	7	87.5±12.5	31	29	93.5±4.4
400	24	22	91.5±5.7	14	13	92.9±7.1	38	35	92.2±4.3
Control	29	27	93.0±4.7	11	11	100.0	40	38	95.0±3.4

The data listed in the table show that the number of males which sired offspring approximates in all the experimental series the value found for the controls. Data on the effectiveness of mating of the females with the male are shown in Table 2.

The data listed in Table 2 as well as those shown in Table 1 support the statement that the first-generation males exhibit normal mating capability, regardless of the dosage of irradiation which we had utilized to treat their sires, and also irrespectively of the time when the litter was sired.

Of special interest is a comparison of the data on the size of the litters sired in the experimental and control series. The data listed in Table 3 reveal that the average number of offspring per litter sired by males, the parent of which was irradiated with 200r or 400r, is less than in the controls. This regularity occurs in the case when the first-generation males were part of a litter sired immediately after the irradiation and also when they were part of a litter sired 3 months after the exposure. Conspicuous is also the fact that the average number of offspring sired by parents irradiated with 400r is lower than in the case of parents irradiated with 200r. However, the difference in this instance is not statistically reliable (M_{dif} = 0.3±0.31), This circumstance permits us to analyze the materials by adding up the data obtained for both exposure dosages. Thus, the average number of offspring per litter sired by males derived from parents immediately after their irradiation amounts to 6.3, whereas in the controls it is 7.0 offspring. The difference thus determined is statistically fully reliable (M_{dif} = 0.7±0.23). At first glance it may appear that decrease of the average size of litters in the experimental series, which is of 0.7 offspring, is very slight and is not worth taking into consideration. Actually, it is very substantial, since it represents 10% of the total number of offspring per litter.

TABLE 2

NUMBER OF EFFECTIVE MATINGS OF FEMALES WITH FIRST-GENERATION MALES*

Irradiation dosage applied to the initial animals (r)	Immediately after irradiation (litters, 1 and 2)				3 months after irradiation (litters 6 and 7)				Total			
	Males tested	Number of matings	Effective		Males tested	Number of matings	Effective		Males tested	Number of matings	Effective	
			Number	Percent			Number	Percent			Number	Percent
200	22	179	112	62.6±3.6	7	54	35	64.8±6.5	29	233	147	63.1±3.2
400	22	127	73	57.5±4.4	13	132	71	53.8±4.3	35	259	144	55.6±3.1
Control	27	160	101	63.0±3.8	11	114	63	55.3±4.6	38	274	164	59.9±2.9

*In contrast with the procedure used in studying the fertility of animals which had been directly subjected to irradiation (see paper by Nuzhdin, N. I., Shapiro, N. I., Petrova, O. N., included in the present symposium) the number of effective matings was computed not for all the males, but only for those which had mated successfully at least once. Males which produced no offspring were not taken into account.

TABLE 3

AVERAGE NUMBER OF OFFSPRING AND NUMBER OF STILLBIRTHS IN LITTERS OF THE SECOND GENERATION

Irradiation dosage applied to initial animals (r)	Time of siring of first generation	Number of males	Number of litters	Number of offspring	Average number of offspring per litter (M±m)	Viable offspring		Stillborn offspring		Average number of viable offspring per litter (M±m)
						Number	Percent	Number	Percent	
200	Immediately after irradiation (litters 1 and 2)	22	105	679	6.5±0.21	666	98.1±0.52	13	1.9±0.52	6.3±0.21
400	same	22	81	503	6.2±0.23	492	97.8±0.65	11	2.2±0.65	6.1±0.24
Total for irradiated	same	44	186	1182	6.3±0.16	1158	98.0±0.41	24	2.0±0.41	6.2±0.16
Control		27	104	725	7.0±0.16	713	98.3±0.47	12	1.7±0.47	6.9±0.17
200	3 months after irradiation (litters 6 and 7)	7	30	197	6.6±0.38	195	99.0±0.71	2	1.0±0.71	6.5±0.42
400	same	13	60	350	5.9±0.29	342	97.7±0.81	8	2.3±0.81	5.8±0.29
Total for irradiated	same	20	90	547	6.0±0.24	537	98.2±0.57	10	1.8±0.57	6.0±0.24
Control		11	59	390	6.6±0.27	383	98.2±0.67	7	1.8±0.67	6.4±0.29

Of importance is also the fact that males sired by irradiated parent 3 months after exposure also show a tendency to sire litters of smaller size. In this instance, the data show that the decrease is due to the males, the parents of which had been irradiated with a dosage of 400r. The difference between average size of litters sired by males, the parents of which had been irradiated with this dosage, and the size of litters sired by the controls is however not reliable statistically ($M_{dif} = 0.7 \pm 0.40$), but still the fact is noteworthy. Thus, the data cited support the statement that decrease of the fertility of first-generation males occurs as a result of a decrease in the number of offspring.

In connection with the decrease in number of the offspring of the first generation, of considerable interest is the question as to whether this decrease is due to a reduction in the number of offspring in the litters sired by all first-generation males, or whether there is some group of individuals which sire litters of appreciably reduced size. To determine this question, an analysis was made of the distribution of first-generation males of the experimental and control series, according to the average number of offspring found in the litters which they have sired. (Table 4).

Figures 1 and 2 show the empirical curves, clearly illustrating the distribution of males depending on the size of their litters, for the experimental and the control groups.

The curves are plotted as follows. The axis of the abscissas represents the average number of offspring per litter, and the axis of ordinates the number of males in percent. The comparison is made for the offspring sired immediately after irradiation (Figure 1) and for that sired 3 months thereafter (Figure 2). The curves characterizing the distribution of the animals of the experimental groups are clearly shifted to the left in comparison with the curves relating to the control groups. Moreover, the nature of these curves indicates the presence among the males of the experimental groups of an excess (in comparison with the controls) of animals of especially low fertility.

TABLE 4

DISTRIBUTION OF FIRST-GENERATION MALES ACCORDING TO THE AVERAGE NUMBER OF OFFSPRING IN THEIR LITTERS

Irradiation dosage applied to the initial animals (r)	Time of siring of the first generation	Males siring average number of offspring per litter	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	Total
200	Immediately after irradiation (litters 1 and 2)	Number	-	1	3	12	4	2	22
		%	-	4.5	13.6	54.6	18.2	9.1	100.0
400	same	Number	1	4	-	13	3	1	22
		%	4.5	18.2	-	59.2	13.6	4.5	100.0
Total for irradiated	same	Number	1	5	3	25	7	3	44
		%	2.3	11.4	6.8	56.8	15.9	6.8	100.0
Control		Number	1	-	-	11	11	4	27
		%	3.7	-	-	40.7	40.7	14.9	100.0
200	3 months after irradiation (litters 6 and 7)	Number	-	-	-	5	2	-	7
		%	-	-	-	71.5	28.5	-	100.0
400	same	Number	1	1	3	6	2	-	13
		%	7.7	7.7	23.1	46.1	15.4	-	100.0
Total for irradiated	same	Number	1	1	3	11	4	-	20
		%	5.0	5.0	15.0	55.0	20.0	-	100.0
Control		Number	-	-	1	5	3	2	11
		%	-	-	9.1	45.4	27.3	18.2	100.0

Thus, the examination of all the above-presented data leaves no doubt of the fact that Xray irradiation causes a decrease of fertility, not only in animals directly subjected to the exposure but also in their offspring.

With the view of a more thorough study of the hereditary nature of Xray induced sterility, we have carried out in addition to the statistical analysis of the phenomenon, also individual genetic analyses of certain first-generation males (sired by an irradiated male by a nonirradiated female). We expected to discover by means of this analysis individual animals of drastically-reduced fertility and to trace the inheritance of this characteristic through a number of later generations.

We took for these tests 3 first-generation males of sharply decreased fertility. One of these (No 1) was sired by a male irradiated with a dosage of 200r, and itself sired on the average 4.3 mice per litter (in six litters altogether). Two other males (No 2 and No 3) belonged to the group of offspring sired by animals irradiated with 400r. One of these two males had sired seven litters numbering on the average 4.9 mice; while the other had sired four litters numbering on the average 4.0 mice.

Tested as to their fertility among the offspring of male No 1, were eight animals of which only one female was found to be semisterile. From this female three litters were obtained numbering on the average 4.3 mice. No further analysis was made of this decrease in fertility. Of the get of male No 2, 18 offspring were tested. Among them were found two females of sharply decreased fertility (respectively 2 and 1 offspring per litter). Analyses of these cases were also limited to production of the second generation. Studied considerably more in detail was the fertility of the offspring of male No 3. The results of this

investigation which encompassed five generations are shown in the graph of Figure 3.

This graph shows clearly the hereditary nature of the sterility exhibited by the first-generation male. The initial male was irradiated with a dosage of 400r. Immediately after the exposure this male sired offspring, including male No 3 (marked in black on the diagram) which was found to be semisterile. The diagram shows the number and size of litters sired by this male as well as by its litter brothers. In the second generation there was found a female of sharply-decreased fertility. In the third generation there was one semisterile female and one entirely sterile (the latter exhibited normal oestrous cycle and was kept for 5 months with males known to be fertile, without producing any offspring), and finally in the fourth generation there were two semisterile males.

Thus, the genetic analysis of a semisterile first-generation male has shown that its descendents exhibit in about 25 to 30% of the cases a sharp reduction in fertility.

The genetic analyses of all three above-mentioned instances of semisterility reveal not only the hereditary nature of the phenomenon under study but also the very nature of the inheritance. As we had pointed out, the Xray semisteriles are associated with chromosome observations of translocation type arising in the genital cells of animals subjected to the exposure. The nature of inheritance of decreased fertility in mice observed in our experiments is in accord with the concept of its translocation origin.

TABLE 5

SEX-RATIOS IN MICE OF SECOND GENERATION DERIVED FROM IRRADIATED (400r) AND NONIRRADIATED ANIMALS

Group of animals	Total number of mice	Including		Males: Females Ratio
		Males	Females	
Experiment	312	155	157	1:1.01
Control	331	165	166	1:1.01

In this connection, we note that inheritance of semisterility occurs according to the dominant type and that animals derived from a semisterile parent, but themselves having a normal fertility, produce in turn only normal offspring.

As was stated hereinbefore, in addition to the fertility of the first-generation individuals, we have investigated the survival, development, and sex-ratios of the second generation. We note that our data indicate an equal survival rate among second generation mice of the experimental and control series. In both cases more than 90% of newborn mice survive to the forty-second day. Of special interest is the determination of sex-ratios in the second generation. In view of the decreased size of litters sired by males of the experimental series, the question arises as to whether this decrease occurs predominantly among the members of one sex. The data obtained (Table 5) leave no doubt that a normal numerical ratio of sexes is found in the second generation.

Study of the development of animals of the second generation was carried out according to the same system as was used for the first generation (see paper by N. I. Nuzhdin, N. I. Shapiro, and O. N. Petrova in the present symposium). Weighing of the mice was done at the following time intervals: on birth, fifth, thirteenth, twenty-first, twenty-eighth,

thirty-fifth, and forty-second day. Since the weight of the animals depends upon sex and size of the litter, the data were processed separately for each sex and litter of definite size. Table 6 shows the data characterizing the development of mice of the second generation.

As is apparent from the data listed in Table 6, the weight of the offspring of second generation derived from irradiated mice practically does not differ from that of the control animals. This equality is found in all the groups being compared and during all the time intervals involved.

(See Table 6 on Page 266)

Especially illustrative is the representation of the dynamics of the changes in weight of the second generation mice shown by the curves of Figure 4.

In conclusion, we note that the occurrence of a normal post-embryonic development of offspring of irradiated animals in the first and the second generation does not mean that exposure to ionizing radiation does not induce, in addition to sterility, hereditary pathological changes of postnatal manifestation. It is merely that the revealing of these changes requires different genetic procedures.

TABLE 6

CHANGES IN THE WEIGHT OF SECOND-GENERATION MICE DERIVED FROM IRRADIATED (400r) AND NON-IRRADIATED ANIMALS

Number of mice per litter	Sex: Males						Females					
	Experiment			Control			Experiment			Control		
	1-3	4-6	7 and more	1-3	4-6	7 and more	1-3	4-6	7 and more	1-3	4-6	7 and more
Number of litters	6	22	23	3	22	26	8	20	23	2	22	26
Weight at birth	1.6	1.4	1.5	1.7	1.6	1.4	1.7	1.4	1.5	1.7	1.6	1.4
Weight at age of 5 days	3.5	2.5	2.6	3.5	2.8	2.5	3.5	2.5	2.6	3.5	2.8	2.5
Weight at age of 13 days	7.5	5.0	4.9	7.1	5.5	4.7	7.8	5.1	5.4	8.4	5.3	4.7
Weight at age of 21 days	11.2	7.5	7.5	10.5	8.3	7.3	10.9	7.8	7.4	11.8	8.3	7.2
Weight at age of 23 days	16.6	11.0	10.8	15.9	11.8	10.7	14.7	11.0	10.8	16.0	11.6	10.4
Weight at age of 35 days	21.3	15.0	14.5	19.1	16.2	14.3	19.0	14.2	14.0	18.5	15.7	13.8
Weight at age of 42 days	23.2	17.4	16.5	22.6	19.1	16.8	19.2	16.2	15.9	-	17.8	15.8

Conclusions

1. Mating capability of first-generation male mice sired by irradiated males does not differ from that of the controls.

2. The number of offspring sired by male mice of the first generation is appreciably decreased in comparison with the controls.

3. There has been ascertained a hereditary nature of the sterility induced by Xray irradiation of mice.

4. Survival, development, and sex-ratios of second-generation animals do not differ from those of the controls.

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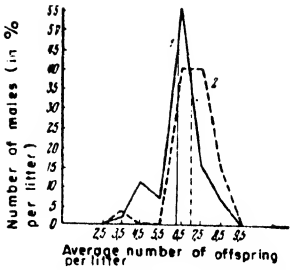

Figure 1. Distribution of first-generation males according to the average number of offspring in their litters (F_1 produced immediately following irradiation).

1, experiment; 2, control.

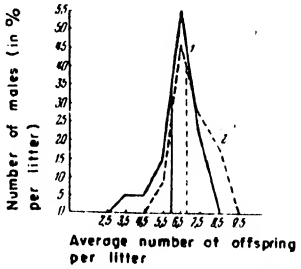

Figure 2. Distribution of first-generation males according to the average number of offspring in their litters (F_1 produced after 3 months following irradiation).

1, experiment; 2, control.

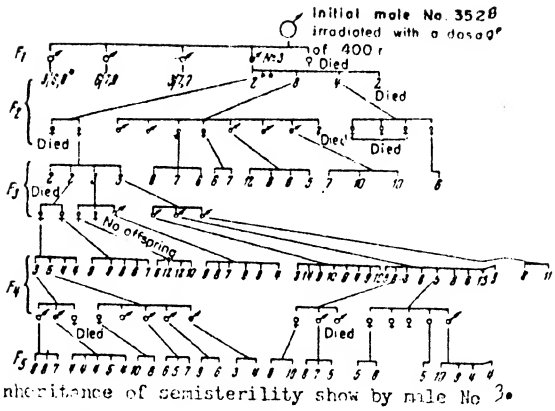

Figure 3. Inheritance of semisterility show by male No 3.

*The numerator of the fraction indicates the number of litters produced, the denominator the average number of offspring per litter.

** Whole numbers indicate the number of offspring in the given litter. Semisteriles are marked in black.

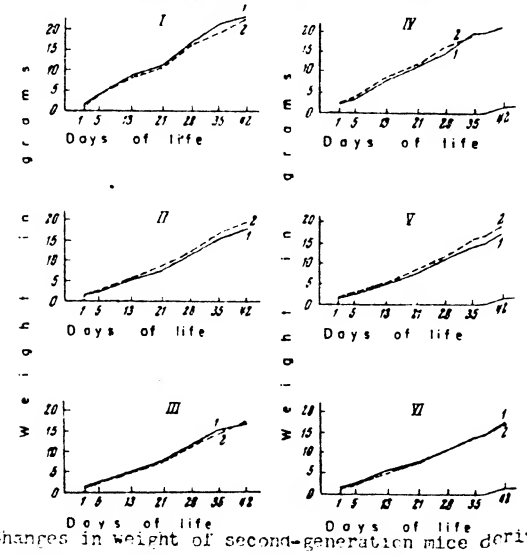

Figure 4. Changes in weight of second-generation mice derived from irradiated males: 1, experiment; 2, control. Males: I - litters of one to 3 offspring; II - litters of 4 to 6 offspring; III - litters of 7 and more offspring. Females: IV - litters of one to 3 offspring; V - litters of 4 to 6 offspring; VI - litters of 7 and more offspring.